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Effect of a plant extract enriched in Stigmasterol and β-Sitosterol on glycaemic status and glucose metabolism in alloxan-induced diabetic rats

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\textbf{Short Title:} Musa antidiabetic potential

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Abstract

Banana is an extensively cultivated plant worldwide, mainly for its fruit, while its ancillary product, the banana pseudostem is consumed as a vegetable and is highly recommended for diabetics in the traditional Indian medicine system. The present study was aimed at elucidating the mechanism of antihyperglycaemia exerted by ethanol extract of banana pseudostem (EE) and its isolated compounds viz., Stigmasterol (C1) and β-Sitosterol (C2) viz., in alloxan-induced diabetic rat model. Diabetic rats which were administered with C1, C2 and EE (100 and 200 mg/kg b. wt.) for 4 weeks showed reduced levels of fasting blood glucose and reversal of abnormalities in serum/urine protein, urea and creatinine in diabetic rats compared to the diabetic control group of rats. Diabetic symptoms such as polyphagia, polydipsia, polyuria, urine glucose and reduced body weight were ameliorated in diabetic group of rats fed with EE, C1 and C2 (100 mg/kg b. wt., once daily) for 28 days. Levels of insulin and Hb were also increased, while the HbA1c level was reduced. The altered activities of hepatic marker enzymes viz., aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP); glycolytic enzyme (hexokinase); shunt enzyme (glucose-6-phosphate dehydrogenase); gluconeogenic enzymes (glucose-6-phosphatase, fructose-1,6-bisphosphatase and lactate dehydrogenase) and pyruvate kinase were significantly reverted to normal levels by the administration of EE, C1 and C2. In addition, increased levels of hepatic glycogen and glycogen synthase and a corresponding decrease of glycogen phosphorylase activity in diabetic rats illustrated the antihyperglycaemic potential of EE and its components. The histological observations revealed a marked regeneration of the β-cells in the drug treated diabetic rats. These findings suggest that EE might exert its antidiabetic potential by the presence of C1 and C2, attributable to the enhanced glycolytic activity, besides increasing the hepatic glucose utilization in diabetic rats by stimulating insulin secretion from the remnant β-cells.
Keywords: Diabetes, ethanol extract, Stigmasterol, β-Sitosterol, glucose metabolism

Introduction

Diabetes mellitus is a non-communicable metabolic disorder caused due to abnormal carbohydrate, protein and lipid metabolism as a result of low blood insulin levels or development of insensitivity of insulin to the target organs. International Diabetes Federation (IDF) predicts an outburst in the number of diabetic patients from 382 million in the year 2013 to 592 million by 2035 of which the major contributors will be China and India.\(^1\) Diabetes represents a heterogeneous complex of disorders all of which leading to persistently higher levels of postprandial blood glucose termed as hyperglycaemic state.\(^2\) Blood glucose homeostasis is chiefly maintained by liver, which involves a coordinated regulation of the carbohydrate metabolic pathways \textit{viz}., glycolysis, pentose phosphate pathway, gluconeogenesis and glycogenolysis.\(^3\) Study of experimental diabetic models have reported that the diabetic state results in increased enzyme activities of gluconeogenic and glycogenolytic pathways and reduced activities in glycolytic and pentose phosphate pathways.\(^4\) The resultant prolonged hyperglycaemia is currently being treated using some of the pharmacological agentssuch as sulfonylurea, biguanide, thiazolidinedione and α-glycosidase inhibitors. Apparently, all these agents have restricted usage due to various undesirable consequences when used for a prolonged period of time.\(^5\)

The complications in the existing diabetic treatment have led to the employment of natural resources either as a food supplement or as a medicinal formulation, to alternate the synthetic drugs. Indian traditional medicine formulates several herbs and is used in the treatment of various diseases since time immemorial.\(^6\) Though they have proved effective, they lack a scientific basis and hence, more recently, a huge research is underway to explain their effectiveness.\(^7\) The expert committees of the World Health Organization (WHO) also
advocate the use of herbal medicines over the drugs currently available for treatment of diabetes which have the potential not only to revert hyperglycaemic state but also reduce the secondary complications associated with it. In this regard, banana (member of Musaceae family) pseudostem, consumed as a vegetable in India, is recommended for diabetics in the Indian traditional medicine system (Ayurveda). In our previous study, the results established ethanol extract of banana pseudostem (EE) as a potent antihyperglycaemic agent and the most prevailing compounds identified (by GC-MS) were phytosterols. The identification of bioactives in EE and its in vivo antidiabetic effects remains to be elucidated. Hence, the present study was designed to investigate the modulatory effects of EE and the compounds isolated from it, on glucose homeostasis in alloxan-induced experimental diabetic rats. The work highlights the effects of EE and its isolated compounds (Stigmasterol and β-Sitosterol) on carbohydrate metabolizing enzymes and on the lipid profile in alloxan induced diabetic rats, thus signifying its efficacy as a prospective intake in diabetic complications.

Materials and methods

Plant Material

Fresh pseudostem of Musa sp. cv. Nanjangud Rasa bale were collected from banana nurturing farms of Nanjangud, Karnataka, India at coordinates 12.11° 7’ 11” North, 76.70° 40’ 58” East. The specimen identification, extraction was performed according to Ramith et al. and the total phenol content (TPC) of the residue was estimated as per Shuxia et al. Isolation and identification of bioactive components

EE was subjected to silica-gel (100-200 mesh) column (length 100 cm and 3 cm diameter) chromatography (elution rate of 2 ml/min flow with a total elution of 600 ml) and eluted with
diverse solvents ranging from non-polar to polar series viz., ether: chloroform (1:1), ethyl acetate, acetone, ethanol, methanol and water. The consequent fractions (Fr) were collected and spotted over pre-coated silica gel F254 plates (20×20 cm, Merck, Germany). The optimum resolution was achieved in the assortment of solvent system hexane, ethyl acetate and formic acid (7.5:2:0.5 v/v) and the plates were sprayed with anisaldehyde-sulphuric acid reagent to visualize the spots. Ether: chloroform (1:1, Fr. I-1-5) fraction had analogous retention factor (Rf) in TLC pattern. Hence, the fractions were pooled and concentrated. The resulting concentrate was re-chromatographed and eluted stepwise with linear gradients of petroleum ether: ethyl acetate (99:1; 98:2; 97:3; 96:4; 95:5; 92.5:7.5; 90:10 v/v). A solitary spot on TLC with suitable solvent system led to the isolation of Stigmasterol and β-Sitosterol from Fr. II (41-45) and Fr. II (46-50) fractions respectively (Fig. 1). It answered Libermann-Burchard’s test for phytosterols.

**Spectral measurements**

The $^1$H (400 MHz) and $^{13}$C NMR (100 MHz) spectra were recorded on a Bruker DRX-400 spectrometer (Bruker Bio spin Co., Karlsruhe, Germany) in CDCl$_3$ with tetramethylsilane (TMS) as an internal standard. The coupling constants in Hz and chemical shifts were expressed in parts per million (δ) relative to the TMS signal. The mass spectrum in ESI mode was obtained using LCMS2010A (Shimadzu, Japan) having probes APCI & ESI. The IR spectra was recorded using KBr discs on a NICOLET 380 FT IR spectrometer (Thermo Fisher Scientific, France) in the range of 400 to 4000nm. The ultraviolet (UV) spectra of the compounds in methanol were recorded on a Shimadzu UV-1800 spectrophotometer. The melting points were determined on an electrically heated VMP-III melting point apparatus and were uncorrected. Further elemental analysis of the compounds was performed on a Perkin Elmer 2400 elemental analyzer.
**Maintenance and usage of RINm5F cell lines**

The RINm5F rat insulinoma cell lines were procured from NCCS, Pune, India. It was cultured in T25 tissue culture flask having RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS) and penicillin G sodium/streptomycin sulphate (100 IU/ml, respectively) in a humidified atmosphere of 5% CO\textsubscript{2} at 37\textdegree C. The optimization of the cell concentration and the insulin secreting assay was carried out as described by Rajesh et al.\textsuperscript{10} All experiments were performed using the cells in the passage 20 in three independent sets.

**In vitro insulin secretion assay**

RINm5F cells were seeded at 1 x 10\textsuperscript{6} cells in 96-well microtitre plate and allowed to adhere for 24 h. The cells were washed with sterile phosphate-buffered saline (PBS, pH 7.2) prior to the incubation (for 45 min) with fresh Krebs-Ringer bicarbonate HEPES (KRBH) buffer comprising of KH\textsubscript{2}PO\textsubscript{4} (1.2 mM), NaHCO\textsubscript{3} (10 mM), NaCl (115 mM), KCl (4.7 mM), CaCl\textsubscript{2} (1.28 mM), MgSO\textsubscript{4}.7H\textsubscript{2}O (1.2 mM) with HEPES (25 mM) added with glucose (1.1 mM) and bovine serum albumin (0.5%, pH 7.4). Subsequently, the cells were treated with five concentrations (7, 16, 25, 50 and 100 µg/ml) of EE and its isolated compounds. After 45 minutes, the buffer was collected and replaced with KRBH buffer supplemented with 4.5 and 16.7 mM glucose. Further, the resulting cells were incubated for 30 minutes in the presence/absence of test samples diluted in KRBH buffer containing 4.5 and 16.7 mM glucose. After incubation, the buffer (supernatant) was removed and the insulin produced was measured by enzyme-linked immunosorbent assay (ELISA) using commercial rat insulin ELISA kit.\textsuperscript{10}
Animals

Healthy adult Wistar rats of either sex (equal number of male and female rats weighing 180-220 g) were obtained from the animal house of JSS College of Pharmacy, Ootacamund, India. The animal experiments were approved by Institutional Animal Ethics Committee (JSSCP/IAEC/Ph.D./PH.COLOGY/01/2013-14) and conducted as per the guidelines of CPCSEA, Chennai, India.²

In vivo Toxicity study

Healthy wistar rats of either sex (equal number of male and female rats weighing 180-220 g) were fasted for 12 hours and then divided into drug-treated ‘test’ groups and vehicle-treated [1% CMC (Carboxy methyl Cellulose)] ‘control’ group, totally making up fourteen groups of six rats each. The ethanol extract of banana pseudostem, Stigmasterol and β-Sitosterol [250, 500, 1000, 2000 mg/kg body weight (b. wt.)] were separately administered to the rats in each of the test groups, while the control group was administered with 1% CMC, to evaluate the toxic effects produced on liver and kidney. Further, the rats in both the testandcontrolgroupswereprovided accessto food and water, and gross behavioural changes were observed over a period of 7 days for signs of acute toxicity.¹¹

Antidiabetic activity of EE, Stigmasterol and β-Sitosterol

Induction of diabetes
Diabetes was induced in the overnight fasted rats weighing 180–220 g by a single intra peritoneal (i.p.) injection of 120 mg/kg alloxan monohydrate dissolved in freshly prepared saline. Animals with fasting blood glucose over 200 mg/dl, 72 h after alloxan injection, were considered diabetic and such animals exhibiting uniform diabetic status were used for further studies. The treatment was initiated on the fourth day after alloxan injection and this day was considered as the first day of treatment.

**Experimental design**

Seven groups of six animals each were segregated for the study as indicated. Group I: served as normal control (1% CMC alone); Group II: diabetic control (1% CMC alone); Group III: diabetic rats + 100 mg/kg b. wt. of EE in 1% CMC; Group IV: diabetic rats + 200 mg/kg b. wt. of EE in 1% CMC; Group V: diabetic rats + 100 mg/kg b. wt. of Stigmasterol in 1% CMC; Group VI: diabetic rats + 100 mg/kg b. wt. of β-Sitosterol in 1% CMC; Group VII: diabetic rats + 250 mg/kg b. wt. of metformin in 1% CMC.

The treatment was carried out up to 28 days. EE, Stigmasterol β-Sitosterol and metformin were dissolved in 1% CMC and administered orally (once a day) by gastric intubation with a force feeding (gauge) needle in a final volume of 1 ml. Fasting blood glucose levels were checked every alternate week using Accu-Chek Blood Glucose Meters (Roche Diagnostics Pvt. Ltd, Mumbai, India). Urine glucose was assessed in the urine samples collected under a layer of toluene by 3, 5-dinitrosalicylic acid method. The body weight, water intake and the dietary intake were observed on day 0, 7, 14, 21 & 28 to evaluate the prevalence of improvement in body weight, polydipsia and polyphagia respectively. These parameters were assessed by shifting rats to the metabolic cages for a period of 24 h. Following the treatment period (i.e. 28 days), the overnight fasted rats were anesthetized using pentobarbitone (30 mg/kg, i.p.) and sacrificed by cervical decapitation during the early hours (between 8:00-
9:00 am). The blood was drawn either from the retro orbital plexus (under mild anaesthesia) during the experiment or from the heart at the time of sacrificing after overnight fasting. It was collected in tubes with/without sodium heparin (20 U/ml blood, in 0.9% saline) for the plasma/ serum separation respectively, followed by centrifugation (2000 rpm for 15 minutes at 40°C). Liver was immediately exercised, washed with chilled physiological saline, homogenised (10 % w/v) with 0.1 M Tris-HCl buffer (pH 7.4) and centrifuged (3000 rpm for 15 minutes at 4°C). The subsequent supernatant was quantified and used for various enzyme assays.

**Biochemical analysis**

On the 0 and 28th day of treatment, haemoglobin content was evaluated by cyanmethaemoglobin method as described by David and Harold,\(^{13}\) and glycated hemoglobin by the method of Nayak and Pattabiraman.\(^{14}\) Also, plasma insulin level was evaluated using DRG Insulin enzyme immunoassay kit (DRG diagnostics, Marburg, Germany) and protein content was estimated by Lowry method.\(^{15}\) The separated serum was assayed for urea, creatinine and uric acid using commercially available diagnostic kit (Span Diagnostics Limited, Surat, India). Subsequently, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were analysed using diagnostic kit (Span Diagnostics Limited, Surat, India). Hepatic glycogen content and activities of glycogen synthase and glycogen phosphorylase were determined according to the methods of Kemp and Adrienne,\(^{16}\) Leloir and Goldemberg,\(^{17}\) and Cornblath et al.\(^{18}\) respectively.

The activities of hepatic carbohydrate metabolic enzymes *viz.*, Glucose-6-phosphate dehydrogenase (EC 1.1.1.49), Fructose-1, 6-bisphosphatase (EC 3.1.3.11) and Glucose-6-phosphatase (EC 3.1.3.9) were performed according to the method of Raju et al.\(^{19}\) Hexokinase (EC 2.7.1.1) assay was performed by the procedures of Mahmood et al.\(^{20}\)
Pyruvate kinase and lactate dehydrogenase activities were measured by the methods of King,\textsuperscript{21} and Pogson and Denton\textsuperscript{22} respectively.

**Histopathological studies**

The pancreas were dissected from the sacrificed animals (one from each group and three samples from each pancreas) cut into smaller pieces (about 1 mm × 1 mm × 1 mm) and fixed with formalin solution (10 %) and immediately processed for histopathological studies by paraffin method. The sections were processed by passing through distinct mixtures of ethyl alcohol and water (45, 75, 95% and incubated in alcohol) for dehydration, cleared in xylene and embedded in paraffin. The ultra-thin sections (5 µm thickness) were achieved using rotary microtome. Further, samples were stained in haematoxylin-eosin (HME) dye and mounted with neutral deparaffinated xylene (DPX) medium for microscopic visualisation. Micrographs (Canon 12.1 mega pixel digital camera, Japan) were captured using Axiostar plus microscope (Zeiss- Germany).\textsuperscript{23}

**Statistical analysis**

Results are expressed as Mean±SD. Statistical comparisons between normal and the treatment groups were performed by one-way analysis of variance (ANOVA), followed by Duncan’s Multiple Range Test using SPSS Software (version 21.0, Chicago, USA). The results were considered statistically significant if the ‘p’ values were 0.05.

**Results**

*Isolation and identification of Stigmasterol and β- Sitosterol from EE*
Repeated chromatographic separations through silica gel column chromatography led to the isolation of Stigmasterol and β- Sitosterol (Fig. 1). The structural elucidation of the compounds was carried out by various physicochemical and spectroscopic methods (UV, IR, 1H NMR, 13C NMR and MS). The details of their structures are as follows,

Stigmasterol (C1) was obtained from Fr. II (41-45) as sticky reddish brown semi solid. m.p.145°C. UV (MeOH): λ max 281 nm. IR (KBr) 3615-3595 cm⁻¹ (O-H).¹¹HNMR (CDCl₃, 400 MHz): δ 0.69, 0.77, 0.85, 0.86, 0.94, 1.04 (each 3H, s, Me × 6), 3.55 (1H, m, H₇3), 5.37 (1H, t, H-6), 5.17 (1H, s, H-22), 5.02 (1H, s, H-23). ¹³C NMR (CDCl₃, 100 MHz): δ 140.7 (C-5), 138.6 (C-22), 129.4 (C-3), 121.8 (C-6), 72.1 (C-3), 57.2 (C-14), 56.2 (C-17), 51.1 (C-24), 50.4 (C-9), 46.7 (C-25), 42.5 (C-13), 40.4 (C-20), 39.9 (C-12), 37.6(C-4), 37.5 (C-1), 36.8 (C-10), 32.6 (C-8), 31.8 (C-7), 29.3 (C-16), 28.5 (C-2), 25.7 (C-28), 24.4 (C-15), 21.3 (C-21), 21.6 (C-11), 20.1 (C-27), 19.3 (C-26), 19.4 (C-19), 12.5 (C-29), 12.6 (C-18). EIMS for C₂₉H₄₈O m/z: 412 [M+1]. Analytical calculated data for (C₂₉H₄₈O): C, 84.40; H, 11.72%. Found C,84.43; H, 11.75%.

β- Sitosterol (C2) was obtained from Fr. II (46-50) as sticky brown semi solid. m.p. 169⁰C. UV (MeOH): λ max 273 nm. IR (KBr) 3600-3580 cm⁻¹ (O-H).¹¹HNMR (CDCl₃, 400 MHz): δ 0.66, 0.79, 0.87, 0.89, 0.94, 1.02 (each 3H, s, Me × 6), 1.26 (4H, q, H-20, 21), 3.58 (1H, m, H-3), 5.36 (1H, t, H-6), 5.18 (1H, s, H-22), 5.05 (1H, s, H-23). ¹³C NMR (CDCl₃, 100 MHz): δ 140.6 (C-5), 138.2 (C-22), 129.3 (C-3), 121.4 (C-6), 72.6 (C-3), 57.5 (C-14), 56.6 (C-17), 51.2 (C-24), 50.4 (C-9), 46.6 (C-25), 42.8 (C-13), 40.5 (C-20), 39.4 (C-12), 37.5(C-4), 37.4 (C-1), 36.7 (C-10), 33.5 (C-20), 32.3 (C-8), 31.4 (C-7), 29.6 (C-21), 29.5 (C-16), 28.4 (C-2), 25.8 (C-28), 24.7 (C-15), 21.8 (C-11), 20.4 (C-27), 19.7 (C-26), 19.4 (C-19), 12.8 (C-29), 12.7 (C-18). EIMS for C₂₉H₅₀O m/z: 414 [M+1]. Analytical calculated data for (C₂₉H₅₀O): C, 83.99; H, 12.15%. Found C, 83.97; H, 12.17%. 
On the basis of the above results, as well in comparison with the NMR and MS data in the literature, compounds (C1 and C2) were identified as Stigmastrolandβ-Sitosterol. These findings also affirm our GC-MS studies that C1 and C2 were the prevailing compounds (present in high amount) in EE. In addition, the total phenolic content of C1 (34.63) and C2 (27.86 mg GAE/g respectively) was found to be high.

**Acute toxicity study of C1 and C2**

Oral administration of EE, C1 and C2 to animals up to a dose of 2000 mg/kg b. wt. did not show any amendment in their behavioural pattern, physical parameters and no animal was dead up to 7 days. In addition, at a dose of 2000 mg/kg b.wt., there was no substantial change in the body weight, water and food intake of the test animals in comparison to the control animals. Further, the animals neither produced any signs of toxicity nor mortality symptoms, thus illustrating the non-toxic nature of EE, C1 and C2. Therefore, further investigation of hypoglycaemic activity was carried out using 100 and 200 mg/kg dose levels.

**In vitro Insulin secretion in RINm5F cells**

The effects of EE and its isolated compounds on insulin secretion (in RINm5F cells) in 4.5 and 16.7 mM glucose concentrations, which represents normal and diabetic conditions respectively, are given in Figure 2A and 2B. A dose dependent increase in insulin secretion was observed at 4.5 and 16.7 mM glucose after 60 minutes treatment with diverse concentrations of EE, Stigmasterol and β - Sitosterol when compared with respective control and tolbutamide treated group.

**Effects of EE and its isolated compounds on diabetic parameters**
The mean fasting blood sugar (FBG) level of normal control rats (group 1) was 79.30 mg/dl in first week and no significant difference was observed until the fourth week (Table 1). The mean of untreated diabetic rats (group 2) increased by 18.1 % (from 295.58 to 395.05 mg/dl at the termination of the experiment), while the mean FBG concentration decreased by 55.13%, 60.99%, 49.31%, 46.22% and 55.88% (for EE 100, EE 200, C1, C2 and metformin respectively) in diabetic treated rats (group 3-7) at the end of 4 weeks. Prior to the administration of the corresponding drugs to all the six diabetic groups (group 2-7), the mean FBG were in the same range. In comparison with diabetic control group, oral administration of EE at two doses (100 and 200 mg/kg b.wt.) and C1 and C2 (100 mg/kg b.wt. respectively) produced a dose dependent decline pattern in FBG concentration till 28 days. Treatment with EE (200 mg/kg b.wt.) caused a maximum and significant reduction in FBG profile after 28 days when compared to metformin (250 mg/kg b.wt.).

The body weight of normal group of rats increased by 8.41% from the initial 187g to 203g at the end of the experimental period. But a major weight loss (-24.15%) was observed in the diabetic control group of rats while the diabetic rats fed with EE (100 and 200 mg/kg b.wt.), C1 and C2 (100 mg/kg b.wt.) showed improvement in the body weight (21.49%, 25.32%, 16.52% and 14.80% respectively) all through the experimental duration (Fig. 3A).

Increased water intake (polydipsia) was witnessed in the diabetes induced group when compared with control group of rats. Oral administration of EE (100 mg/kg b.wt.), EE (200 mg/kg b.wt.), C1 and C2 (100 mg/kg b.wt.) to diabetic treated group of rats showed a reduction in water consumption (-26.32%, -30.22%, -24.70% and -23.47% respectively) at the end of 4 weeks (Fig. 3B).

As shown in Figure 3C, all the six diabetic group of rats exhibited polyphagia after the injection of alloxan compared with normal control group of rats. However, the diet
consumption reduced in diabetic treated group of rats (group 3-7) compared to diabetic control rats (group 2).

The augmented level of urine excretion during diabetes was monitored every alternate week. Urine volumes from diverse group of rats presented in Figure 3D, illustrate that diabetic rats excreted more than 3.5 folds higher volumes of urine than normal control rats. The polyuria state of the rats treated with EE 100, EE 200, C1 and C2 (-4.18%, -4.67%, -3.42% and -3.06% respectively) decreased progressively all through the experiment in comparison with the diabetic control rats.

The urinary excretion of various metabolites viz., sugar, protein, urea and creatinine was measured on day 0 and 28. Normal control group of rats excreted reducing sugar in milligram quantities, whereas urinary excretion of sugar by the diabetic rats remained high all through the experiment. Along with the excretion of a lower urine volume, the diabetic treated group of rats exhibited a significant reduction in glucosuria on the last day, compared to first day of the experiment (Table 2).

Increased protein, urea and creatinine excretion in the urine was observed in all the six groups of diabetes induced rats prior to the drug administration. These criterions improved to near normal upon treatment with EE (100 and 200 mg/kg b.wt.), C1 (100 mg/kg b.wt.), C2 (100 mg/kg b.wt.) and metformin(250 mg/kg b.wt.), from the initial day to the end of the experiment (day 28). The values of five diabetic groups were significantly lower compared to the diabetic control group and on par with the normal group of rats.

In addition, the diabetic state lead to a decrease in total serum protein and an increase in urea and creatinine levels (Table 2). Oral administration of EE, C1, C2 and the positive control (metformin) to diabetic rats exhibited improved total protein levels to near normal by day 28.
The levels of urea and creatinine in the serum were decreased to near normal, compared to those of the diabetic control group.

Assessment of plasma insulin, hemoglobin (Hb) and glycated hemoglobin (HbA1c)

In comparison with the normal control group of rats, a reduced level of plasma insulin and Hb along with a higher level of HbA1c was evident in the alloxan-induced diabetic rats (Table 3). However at the end of the experiment, these levels were ameliorated towards near normal on treatment with EE(100 and 200 mg/kg b.wt.), C1(100 mg/kg b.wt.), C2(100 mg/kg b.wt.) and metformin(250 mg/kg b.wt.) drugs. The treatment significantly reversed the upraised levels of HbA1c and resumed the levels of plasma insulin and Hb to normal levels in the alloxan-induced diabetic rats.

Activities of serum AST, ALT and ALP and hepatic glycogen and its metabolic enzymes

Table 4A presents the effect of EE(100 and 200 mg/kg b.wt.), C1(100 mg/kg b.wt.), C2(100 mg/kg b.wt.) and metformin(250 mg/kg b.wt.) on activities of serum AST, ALT and ALP in normal and alloxan-induced diabetic rats after 28 days of treatment. In diabetic rats, there was a significant (p ≤0.05) increase in the activities of AST (3 fold), ALT (1.8 fold) and ALP (2 fold) when compared to the normal control group. Oral administration of EE and its constituents, significantly (p ≤0.05) decreased the activities when compared to the diabetic control rats.

Table 4B presents the hepatic glycogen metabolism of the control and experimental animals. A significant deterioration in the glycogen level along with the glycogen synthase activity and an allied increase in the activity of glycogen phosphorylase were observed in the liver of
diabetic control group of rats. Oral administration of EE (100 and 200 mg/kg b.wt.), C1 and C2 (100 mg/kg b.wt.) to the diabetic rats for 28 days, exhibited a significant decrease in the glycogen phosphorylase activity and an increase in the glycogen level as well as glycogen synthase activity when compared to the diabetic control rats.

**Estimation of activities of carbohydrate metabolic enzymes**

Table 5 illustrates the effect of the oral administration of EE (100 and 200 mg/kg b.wt.), C1 and C2 (100 mg/kg b.wt.) for 28 days on carbohydrate metabolic enzymes in the liver of control and experimental animals. Diabetic control group of rats showed deteriorated activities of hexokinase, glucose-6-phosphate dehydrogenase and pyruvate kinase, whereas a concomitant increase in the activities of the gluconeogenic enzymes (glucose-6-phosphatase, fructose-1,6-bisphosphatase and lactate dehydrogenase) in the liver. Oral administration of EE (100 and 200 mg/kg b.wt.), C1 and C2 (100 mg/kg b.wt.) reinstated the level of gluconeogenic enzymes and the activities of key enzymes of the carbohydrate metabolism to near normal when compared to normal control rats.

For all the biochemical parameters studied above, oral administration of EE (100 and 200 mg/kg b.wt.), C1 and C2 (100 mg/kg b.wt.) for 28 days to the diabetic rats showed significant effects in comparison with the control rats. Nevertheless, EE at 200 mg/kg b.wt. showed the maximum effect and was significantly higher (p < 0.05) than metformin (250 mg/kg b.wt.). In contrast, C1 and C2 (100 mg/kg b.wt.) also brought back all the parameters to near normal and the values were comparable to those of metformin.

**Histological investigations of pancreas**
Histology of pancreas section of one rat from each group is depicted in Fig. 4A-G and these effects corroborated with our biochemical outcomes. Histopathological examination illustrated that alloxan administration caused spartan damage to pancreatic β-cells such as changes in dilation and de-granulation of islet cells besides decrease in number (Fig. 4B). The pancreas of normal control group showed the normal architecture, consisting of normal lobules/acini (exocrine portion) and normal islets of Langerhans (endocrine portion) with well organised α (alpha), β (beta) and δ (delta) cells (Fig. 4A). Oral administration of EE (100 and 200 mg/kg b.wt.), C1(100 mg/kg b.wt.), C2(100 mg/kg b.wt.) and metformin(250 mg/kg b.wt.) for 28 days to diabetic rats rejuvenated the normal cellular population with an increase in the islets in comparison to the diabetic control group (Fig. 4C-G).

Discussion

Plant based therapeutics have paved new avenues in the pharmaceutical research because of the successful isolation and identification of several bioactive compounds which are regarded safe as against the currently available synthetic drugs, which are known to be associated with several deleterious effects. With this background, Ethanol extract of the banana pseudostem (EE) and the phytosterols (Stigmasterol and β-Sitosterol) isolated from EE were evaluated for their role on the glucose utilization and on hepatic glycogen degradation pathways which are the primary contributors of hyperglycaemia. The studies were carried out using the alloxan-induced diabetic rats in which, the administration of alloxan led to a massive destruction of the β-cells of the islets of Langerhans. The resulting impairment in the insulin secretion disarrayed the regulation of plasma glucose, in turn, leading to hyperglycaemia. Epidemiological studies uphold the concept of hyperglycaemia as the key factor resulting in
an array of diabetic complications\textsuperscript{6} and hence the role of EE was primarily tested on the reduction of plasma glucose levels in the diabetic models. Ethanol extract of pseudostem (100 and 200 mg/kg b.wt.), Stigmasterol and β-Sitosterol (100 mg/kg b.wt.) exhibited positive effects by decreasing the plasma glucose levels which could possibly be due to either of the two mechanisms or a collective effect of both. i.e., it could be because of the reduction in the intestinal glucose absorption\textsuperscript{2} or elevation in the glycolytic and glycogenic pathways with a concomitant decrease in the glycogenolysis and gluconeogenesis pathways which suggests its effects on the glucose metabolic pathways.\textsuperscript{27}

Other mechanism for the antihyperglycaemic effect could be the stimulation or regeneration of the β-cells and a subsequent secretion of insulin or activation of the insulin receptors.\textsuperscript{28} The treatment with EE (100 and 200 mg/kg b.wt.) demonstrated a significant elevation in the insulin levels and a corresponding increase in the amount of insulin-secreting cells in many parts of the pancreatic islets. A significantly promising result was also observed upon administration of Stigmasterol and β-Sitosterol (100 mg/kg b.wt.) which was on par with the standard antidiabetic drug metformin (250 mg/kg b.wt.) in improving the glycaemic regulation and promoting the secretion of insulin from the remnant β-cells. In addition, to assess the functions of the pancreas, \textit{in vitro} glucose-induced insulin secretion study was also performed, using rat insulinoma cells (RINm5F). The \textit{in vitro} study illustrated an insulinotropic activity by enhanced insulin secretion after treatment with EE and the compounds at both 4.5 and 16.7 mM glucose concentrations. Numerous studies have reported the beneficial role of β-Sitosterol and Stigmasterol in stimulating the insulin secretion thereby possessing an antihyperglycaemic effect.\textsuperscript{29-30} Our studies previously also report the abundance of these phytosterols in EE, which promotes their use as dietary supplements for diabetic patients.\textsuperscript{2}
Diabetes also results in polyuria, polyphagia and polydipsia which are collectively caused during insulin deficit and a concomitant hyperglycaemia resulting from it. In this state, the catabolism of protein is stimulated to supplement the amino acids required for gluconeogenesis, which reflects as the loss of structural proteins, leading to the muscle wasting and weight loss. In our study, the increased food and fluid consumption exhibited by the alloxan-induced diabetic rats were reverted by the oral treatment of EE(100 and 200 mg/kg b.wt.), Stigmasterol and β-Sitosterol (100 mg/kg b.wt.) which could be due to the improvement in the glucose metabolism. The differences in the body weight in course of the experimental period may be attributed to the protective role of EE, Stigmasterol and β-Sitosterol against the gluconeogenesis-induced muscle wasting and the improved glycaemic control.

Another concern during diabetes is the renal alterations which are a resultant of the persistently high plasma glucose levels and a concomitantly high protein metabolism, which leads to a negative nitrogen balance characterized by the elevated urea and creatinine levels. These serve as the markers for renal dysfunction and generally high both in serum and urine during diabetes. There was a noteworthy reduction in the levels of these parameters after treatment with EE and the isolated phytosterols (Stigmasterol and β-Sitosterol), establishing their protective role in terms of the renal alternations during glucose toxicity. While the catabolism of proteins is one effect of hyperglycaemia, glycation of proteins including hemoglobin (Hb), albumin, collagen, low-density lipoprotein is its other effect. A significant increase in the level of HbA1c is witnessed during diabetes and its levels prove as a reliable indicator of the glycaemic control during its management. Oral administration of EE, Stigmasterol and β-Sitosterol effectively reduced the levels of HbA1c and increased the levels of Hb which indicate an optimal control of glucose levels in the blood.
The excess glucose is stored in the form of glycogen chiefly in the liver and skeletal muscles. Cells employ insulin to stimulate glycogen synthase and inhibit glycogen phosphorylase, which will in turn promote glycogen synthesis while inhibiting the glycogen breakdown respectively. However, diabetic conditions lead to a marked decrease in the levels of glycogen in the skeletal muscle and liver, which again is a resultant of the impaired insulin secretion. Also, a substantial reduction in the liver weight due to the increased breakdown of glycogen, protein degradation and increased gluconeogenesis is witnessed. These anomalies are corrected in the EE, Stigmasterol and β-Sitosterol treated rats which are in agreement with the studies of Rangachari et al., by regulating the glycogen metabolizing enzymes to near normal levels.

Furthermore, there exists a marked increment in the plasma levels of AST, ALT and ALP during diabetes which fundamentally is due to the leakage of these enzymes from the cytosol of the liver into the blood. These enzymes are reliable markers for hepatic dysfunction which also represents the toxic effects of alloxan on the liver as previously proven by Larcan et al. who reported a necrotized liver in diabetic patients. Yet, the treatment of EE, Stigmasterol and β-Sitosterol brought down the levels of these enzymes which suggest their hepatoprotective role during hepatotoxicity induced by hyperglycaemia.

Though hyperglycaemia is primarily caused by reduced glycolysis and hepatic glycogen storage whilst an increased hepatic glucose production, a complex of various enzymes involved in the glucose storage and glycogen degradation pathway tightly regulate these processes. Glycolysis is initiated by the key enzyme hexokinase, an insulin dependent enzyme, which phosphorylates glucose to glucose-6-phosphate. The levels of hexokinase are distinctly reduced during diabetes which reflects on the reduced glucose utilization by the tissues. Nevertheless, the treatment with EE, Stigmasterol and β-Sitosterol raised the levels of this rate limiting enzyme of the glycolysis, proportionally to the levels of insulin.
confirms the improvement of hexokinase levels because of the increase in the insulin levels which stimulated better glucose utilization by the hepatic cells.

Also, other enzymes namely lactate dehydrogenase (LDH) and pyruvate kinase (PK) with the terminal glycolytic enzymes, play chief role in regulating glycolysis. LDH catalyzes the interconversion of lactate to pyruvate, producing energy under anaerobic conditions. The reaction can occur both in cytosol and mitochondria and known to be dependent on insulin, glucose and NADH concentrations. Likewise, PK catalyzes the conversion of phosphoenol pyruvate to pyruvate and the resulting pyruvate from both the enzyme catalyzed reactions feed the substrate to the citric acid cycle. The two enzymes are activated by insulin and hence, diabetic conditions also show a reduced level of these glycolytic enzymes. Similar to the upregulation of hexokinase, the treatment of EE resulted in an enhancement in the activity of LDH and PK which shows up on the increased glycolysis (or increased utilization of glucose).

Another significant contributor for the plasma glucose level is the gluconeogenic pathway of which the two enzymes glucose-6-phosphatase and fructose-1, 6-bisphosphatase catalyze the regulatory steps. Though diabetic state is characterized by the high levels of plasma glucose, their availability to the tissues are restricted. This activates the gluconeogenic enzymes and hence their levels remain high for a prolonged period in diabetes. The results of our study expressed a significant drop of these enzymes which probably is due to the metabolic control that was taken over by insulin after EE treatment in the alloxan induced diabetic rats.

Glucose levels are also affected by the pentose phosphate pathway which is essential for the production of pentose sugar, ribose-5-phosphate and the reducing equivalent NADPH. The regulatory enzyme, glucose-6-phosphate dehydrogenase is the principle control step in the pathway and hence its low levels lead to oxidative damage through lower levels of NADPH.
which is the case during diabetes. However, the EE, Stigmasterol and β-Sitosterolefficiently raised the levels of this enzyme and hence extended a protective role against the induced oxidative damage (by hydrogen donation to NADP+ to produce NADPH) and augmented the synthesis of fats from carbohydrates (lipogenesis) resulting in reduced plasma glucose concentration.

**Conclusion**

Administration of EE, Stigmasterol and β-Sitosterol, restored the β-cells thereby stimulating them to produce insulin. The improved levels of insulin also positively promoted the activities of the glucose utilization and the glycogen storage pathways indicated by the measurement of insulin levels and also by the antihyperglycaemic effects, as well as the analysis of various metabolic enzymes involved in the pathways. These results are promising in considering EE and its constituents Stigmasterol and β-Sitosterol as potential antidiabetic herbal remedies in the management of diabetes and its associated complications.

**Acknowledgement**

All authors thank Dr. B. Yashovarma, Secretary, SDM educational society for his support and encouragement. The authors also thank The Principal, SJCE, Mysore for his encouragement. DBL would like to place on record the deepest gratitude towards Dr. Chenraj Roychand, President, Jain University trust and Dr. Krishna Venkatesh, CEO, Centre for Emerging Technologies (CET) for their constant motivation and support.

**Conflict of interest**

The authors declare there is no conflict of interest
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Figure 1. Separation scheme of active compounds from ethanol extract of banana pseudostem and structures of isolated compounds.

Figure 2. The effect of diverse concentrations of ethanol extract of pseudostem (EE), Stigmasterol (C1), β – Sitosterol (C2) and tolbutamide (positive control) on insulin secretion in RINm5F cells in the presence of 4.5 mM (A) and 16.7 mM (B) glucose load. Data are expressed as the mean± SD, n = 3 of independent experiments. Means in the concentrations (7, 14, 25, 50 & 100) with distinct superscripts are significantly different (p ≤0.05) as separated by Duncan multiple range test.

Figure 3. Effect of ethanol extract of pseudostem (EE) and its isolated compounds on (A) body weight (grams), (B) water intake (ml/24 hours), (C) diet intake (grams/24 hours) and (D) urine volume (ml/24 hours) in control and diabetic rats. Parameters were monitored at weekly intervals (after 0, 14, 21, 28 days of treatment). Data are expressed as the mean± SD. Means in the weeks (1, 2, 3 & 4) with distinct superscripts are significantly different (p ≤0.05) as separated by Duncan multiple range test. Abbreviations and ‘n’ values are as defined in Table 2.

Figure 4. Histopathological examinations of pancreas in experimental rats after 28 days of treatment with ethanol extract of pseudostem (EE) and its isolated compounds Stigmasterol (C1) & β – Sitosterol (C2) (H and E, 40 x). Micrographs showing normal (A) intact islets with cellular characteristics of pancreas; diabetic group (B) with degeneration of pancreatic acini, apathy of β cells (necrotic and fibrotic changes) and emaciated islets of Langerhans; EE
100 (C), EE 200 (D), C1 (E), C2 (F) and metformin (G) with refurbishment of normal cellular population of pancreatic acini/lobules and with size, shape of the islets. Abbreviations are as defined in Table 2.
Table 1. Effect of ethanol extract of pseudostem (EE) and its isolated compounds on fasting blood glucose levels in normal experimental diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose level x (mg/dl)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Normal control</td>
<td>79.30±3.36 a</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>295.58±8.41 b, c</td>
</tr>
<tr>
<td>Diabetic + EE (100 mg/kg b. wt.)</td>
<td>301.33±15.45 b, c</td>
</tr>
<tr>
<td>Diabetic + EE (200 mg/kg b. wt.)</td>
<td>288.35±18.23 b</td>
</tr>
<tr>
<td>Diabetic + Stigmasterol (100 mg/kg b. wt.)</td>
<td>304.01±13.56 c</td>
</tr>
<tr>
<td>Diabetic + β-Sitosterol (100 mg/kg b. wt.)</td>
<td>294.68±7.07 b, c</td>
</tr>
<tr>
<td>Diabetic + Metformin (250 mg/kg b. wt.)</td>
<td>287.35±4.61 b</td>
</tr>
</tbody>
</table>

x Values are expressed as mean ± SD with six animals per group (n=6). Means in the same column with distinct superscripts are significantly different (p ≤0.05) as separated by Duncan multiple range test.
Table 2. Effect of ethanol extract of pseudostem (EE) and its isolated compounds on urinary excretion of (a) sugar, protein, urea and creatinine (mg/24h respectively) and (b) serum protein, urea and creatinine (mg/24h respectively) in normal and experimental diabetic rats.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Group 1 (normal)</th>
<th>Group 2 (diabetic)</th>
<th>Group 3 (EE 100)</th>
<th>Group 4 (EE 200)</th>
<th>Group 5 (C1)</th>
<th>Group 6 (C2)</th>
<th>Group 7 (metformin)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urine</strong></td>
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<tr>
<td>Glucose (mg/24h)</td>
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<tr>
<td>Day 0</td>
<td>3.17±1.19</td>
<td>7867.93±9.20</td>
<td>6873.67±14.06</td>
<td>6996.06±7.79</td>
<td>7353.83±15.07</td>
<td>7576.33±12.91</td>
<td>7652.77±16.47</td>
</tr>
<tr>
<td>Day 28</td>
<td>2.59±6.69</td>
<td>9112.83±2.31</td>
<td>2882.15±8.78</td>
<td>2656.70±8.21</td>
<td>3165.67±11.27</td>
<td>3342.27±13.81</td>
<td>3072.44±13.14</td>
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<tr>
<td>Protein (mg/24h)</td>
<td></td>
<td></td>
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<tr>
<td>Day 0</td>
<td>3.51±1.07</td>
<td>24.80±3.31</td>
<td>21.82±1.72</td>
<td>24.77±2.66</td>
<td>24.83±2.74</td>
<td>22.10±1.82</td>
<td>21.31±3.73</td>
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<tr>
<td>Day 28</td>
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<td>10.91±1.70</td>
<td>10.50±2.01</td>
<td>12.90±2.20</td>
<td>11.77±3.13</td>
<td>10.34±0.83</td>
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<tr>
<td>Urea (mg/24h)</td>
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<tr>
<td>Day 0</td>
<td>25.34±1.16</td>
<td>246.47±4.67</td>
<td>221.82±1.72</td>
<td>224.77±2.66</td>
<td>224.83±2.74</td>
<td>213.60±3.62</td>
<td>221.31±3.73</td>
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<tr>
<td>Day 28</td>
<td>26.09±1.03</td>
<td>328.50±2.19</td>
<td>134.25±3.99</td>
<td>123.50±7.13</td>
<td>139.56±4.32</td>
<td>144.44±6.27</td>
<td>122.00±4.00</td>
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<tr>
<td>Creatinine (mg/24h)</td>
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<tr>
<td>Day 0</td>
<td>6.08±2.01</td>
<td>31.19±2.80</td>
<td>29.10±3.53</td>
<td>29.73±3.36</td>
<td>29.13±3.82</td>
<td>32.58±3.48</td>
<td>31.23±2.79</td>
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<tr>
<td>Day 28</td>
<td>6.09±2.44</td>
<td>39.49±3.62</td>
<td>20.13±2.98</td>
<td>19.88±3.43</td>
<td>20.52±4.00</td>
<td>18.67±3.77</td>
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<tr>
<td><strong>Serum</strong></td>
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<td>Protein (mg/24h)</td>
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<tr>
<td>Day 0</td>
<td>7.45±2.61</td>
<td>5.27±1.62</td>
<td>5.49±1.45</td>
<td>5.24±1.28</td>
<td>5.11±1.53</td>
<td>5.09±0.97</td>
<td>5.40±0.67</td>
</tr>
<tr>
<td>Day 28</td>
<td>7.97±2.72</td>
<td>4.28±1.22</td>
<td>6.90±0.91</td>
<td>6.80±1.60</td>
<td>6.49±2.59</td>
<td>6.12±1.57</td>
<td>6.66±1.50</td>
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<tr>
<td>Urea (mg/24h)</td>
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<tr>
<td>Day 0</td>
<td>28.62±3.11</td>
<td>54.93±8.19</td>
<td>55.66±8.70</td>
<td>51.37±6.29</td>
<td>51.50±3.50</td>
<td>50.09±3.80</td>
<td>50.33±3.98</td>
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<tr>
<td>Day 28</td>
<td>29.71±2.01</td>
<td>63.16±5.58</td>
<td>40.32±4.46</td>
<td>36.42±1.80</td>
<td>38.91±4.13</td>
<td>39.20±2.86</td>
<td>37.67±4.68</td>
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<tr>
<td>Creatinine (mg/24h)</td>
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<tr>
<td>Day 0</td>
<td>0.43±0.11</td>
<td>0.96±0.11</td>
<td>0.91±0.10</td>
<td>0.91±0.13</td>
<td>0.92±0.13</td>
<td>1.01±0.08</td>
<td>0.98±0.12</td>
</tr>
<tr>
<td>Day 28</td>
<td>0.45±0.21</td>
<td>1.42±0.14</td>
<td>0.68±0.12</td>
<td>0.62±0.08</td>
<td>0.70±0.12</td>
<td>0.75±0.13</td>
<td>0.71±0.17</td>
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</tbody>
</table>

Normal: normal control; diabetic: diabetic control; EE 100: Diabetic + EE (100 mg/kg b. wt.); EE 200: Diabetic + EE (200 mg/kg b. wt.); C1: Diabetic + Stigmasterol (100 mg/kg b. wt.); C2: Diabetic + β- Sitosterol (100 mg/kg b. wt.); metformin: Diabetic + metformin (250 mg/kg b. wt.).

Values are expressed as mean ± SD with six animals per group (n=6). Means in the same row with distinct superscripts are significantly different (p ≤0.05) as separated by Duncan multiple range test.
Table 3. Levels of plasma insulin, hemoglobin (Hb) and glycated hemoglobin (HbA1c) of normal and experimental diabetic rats after 0 and 28 days of treatment with ethanol extract of pseudostem (EE) and its isolated compounds

<table>
<thead>
<tr>
<th></th>
<th>Insulin (µU/ml)</th>
<th>Hb (g/dl)</th>
<th>HbA1c (%Hb)</th>
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<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 28</td>
<td>Day 0</td>
</tr>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(normal)</td>
<td>17.10±1.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.03±1.53&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.70±1.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(diabetic)</td>
<td>8.87±1.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.24±1.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.17±1.18&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td><strong>Group 3</strong></td>
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<tr>
<td>(EE 100)</td>
<td>8.54±1.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.99±1.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.31±0.98&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td><strong>Group 4</strong></td>
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<tr>
<td>(EE 200)</td>
<td>8.98±1.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.00±1.93&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>8.10±0.77&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td><strong>Group 5</strong></td>
<td></td>
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<tr>
<td>(C1)</td>
<td>8.82±1.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.90±1.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.22±0.91&lt;sup&gt;a&lt;/sup&gt;</td>
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<td><strong>Group 6</strong></td>
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<tr>
<td>(C2)</td>
<td>9.50±1.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.17±2.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.40±1.17&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td><strong>Group 7</strong></td>
<td></td>
<td></td>
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<tr>
<td>(metformin)</td>
<td>8.39±1.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.21±2.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.32±1.36&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. Means in the same column with distinct superscripts are significantly different (p ≤0.05) as separated by Duncan multiple range test. Abbreviations and ‘n’ values are as defined in Table 2.
Table 4. Effect of ethanol extract of pseudostem (EE) and its isolated compounds on activities of (a) serum AST, ALT and ALP and (b) hepatic glycogen content, activities of glycogen synthase and glycogen phosphorylase.

<table>
<thead>
<tr>
<th>Group</th>
<th>Group 1 (normal)</th>
<th>Group 2 (diabetic)</th>
<th>Group 3 (EE 100)</th>
<th>Group 4 (EE 200)</th>
<th>Group 5 (C1)</th>
<th>Group 6 (C2)</th>
<th>Group 7 (metformin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a AST</td>
<td>28.84±2.32</td>
<td>103.31±3.43</td>
<td>44.37±5.45</td>
<td>35.88±5.49</td>
<td>50.80±3.38</td>
<td>53.52±11.18</td>
<td>48.65±3.78</td>
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<tr>
<td>ALT</td>
<td>11.94±1.62</td>
<td>21.93±2.77</td>
<td>15.20±2.46</td>
<td>13.59±3.17</td>
<td>17.88±1.67</td>
<td>17.86±0.75</td>
<td>14.54±1.77</td>
</tr>
<tr>
<td>ALP</td>
<td>31.21±3.19</td>
<td>60.67±7.76</td>
<td>35.16±4.96</td>
<td>32.88±5.20</td>
<td>41.33±8.09</td>
<td>39.83±5.71</td>
<td>38.33±6.05</td>
</tr>
<tr>
<td>b Glycogen</td>
<td>52.70±3.03</td>
<td>16.99±1.43</td>
<td>40.41±3.36</td>
<td>45.52±2.49</td>
<td>37.83±6.91</td>
<td>34.11±1.77</td>
<td>41.33±1.97</td>
</tr>
<tr>
<td>Glycogen synthase</td>
<td>805.81±5.61</td>
<td>477.87±9.26</td>
<td>738.50±13.46</td>
<td>762.23±17.47</td>
<td>726.17±10.65</td>
<td>717.83±4.62</td>
<td>728.35±20.03</td>
</tr>
<tr>
<td>Glycogen phosphorylase</td>
<td>608.50±9.48</td>
<td>809.00±10.04</td>
<td>714.17±12.72</td>
<td>676.67±15.58</td>
<td>727.33±9.77</td>
<td>732.50±19.69</td>
<td>693.56±16.06</td>
</tr>
</tbody>
</table>

AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase.

* (w): IU/L; (x): mg/g tissue; (y): µmoles of UDP formed/hour/mg protein; (z): µmoles of Pi liberated/hour/mg protein.

Values are expressed as mean ± SD. Means in the same row with distinct superscripts are significantly different (p ≤0.05) as separated by Duncan multiple range test. Abbreviations and ‘n’ values are as defined in Table 2.
Table 5
Activities of hepatic key enzymes of carbohydrate metabolism in normal and experimental diabetic rats after 28 days of treatment with ethanol extract of pseudostem (EE) and its isolated compounds

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (normal)</th>
<th>Group 2 (diabetic)</th>
<th>Group 3 (EE 100)</th>
<th>Group 4 (EE 200)</th>
<th>Group 5 (C1)</th>
<th>Group 6 (C2)</th>
<th>Group 7 (metformin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexokinase <em>w</em></td>
<td>0.26±0.02 a</td>
<td>0.09±0.03 a</td>
<td>0.23±0.05 c, d</td>
<td>0.24±0.05 c, d</td>
<td>0.19±0.01 b</td>
<td>0.20±0.02 b, c</td>
<td>0.21±0.04 b, c</td>
</tr>
<tr>
<td>Glucose-6-phosphatase x</td>
<td>0.14±0.08 a</td>
<td>0.33±0.06 b</td>
<td>0.18±0.08 a</td>
<td>0.16±0.20 a</td>
<td>0.20±0.10 a</td>
<td>0.19±0.05 a</td>
<td>0.18±0.11 a</td>
</tr>
<tr>
<td>Fructose-1,6-bisphosphatase x</td>
<td>0.07±0.05 a</td>
<td>0.26±0.08 c</td>
<td>0.14±0.06 a, b</td>
<td>0.12±0.06 a, b</td>
<td>0.16±0.05 b</td>
<td>0.17±0.04 b</td>
<td>0.11±0.02 b</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase y</td>
<td>3.40±0.09 f</td>
<td>2.21±0.08 a</td>
<td>3.01±0.07 d</td>
<td>3.16±0.05 e</td>
<td>2.85±0.05 b, c</td>
<td>2.90±0.07 c</td>
<td>2.80±0.10 b</td>
</tr>
<tr>
<td>Lactate dehydrogenase z</td>
<td>242.38±10.50 a</td>
<td>454.31±6.68 d</td>
<td>283.29±5.83 c</td>
<td>270.19±7.01 b</td>
<td>288.06±9.43 c</td>
<td>292.32±7.73 c</td>
<td>270.54±8.64 b</td>
</tr>
<tr>
<td>Pyruvate kinase z</td>
<td>187.80±7.47 e</td>
<td>88.58±5.50 a</td>
<td>153.69±5.09 b, c</td>
<td>161.76±7.30 d</td>
<td>158.90±7.31 c, d</td>
<td>146.96±5.52 b</td>
<td>149.01±4.95</td>
</tr>
</tbody>
</table>

* (w): µmoles of glucose phosphorylated/h/mg protein; (x): µmoles of Pi liberated/h/mg protein; (y): U/mg protein; (z): µmoles of Pyruvate formed/h/mg protein. Values are expressed as mean ± SD. Means in the same row with distinct superscripts are significantly different (p ≤0.05) as separated by Duncan multiple range test. Abbreviations and ‘n’ values are as defined in Table 2.
Figure 1. Separation scheme of active compounds from ethanol extract of banana pseudostem and structures of isolated compounds.

254x190mm (96 x 96 DPI)
Figure 2. The effect of diverse concentrations of ethanol extract of pseudostem (EE), Stigmasterol (C1), β-Sitosterol (C2) and tolbutamide (positive control) on insulin secretion in RINm5F cells in the presence of 4.5mM (A) and 16.7 mM (B) glucose load. Data are expressed as the mean± SD, n = 3 of independent experiments. Means in the concentrations (7, 14, 25, 50 & 100) with distinct superscripts are significantly different (p ≤0.05) as separated by Duncan multiple range test.
Figure 3. Effect of ethanol extract of pseudostem (EE) and its isolated compounds on (A) body weight (grams), (B) water intake (ml/24 hours), (C) diet intake (grams/24 hours) and (D) urine volume (ml/24 hours) in control and diabetic rats. Parameters were monitored at weekly intervals (after 0, 14, 21, 28 days of treatment). Data are expressed as the mean±SD. Means in the weeks (1, 2, 3 & 4) with distinct superscripts are significantly different (p ≤0.05) as separated by Duncan multiple range test. Abbreviations and ‘n’ values are as defined in Table 2.

254x190mm (96 x 96 DPI)
Figure 4. Histopathological examinations of pancreas in experimental rats after 28 days of treatment with ethanol extract of pseudostem (EE) and its isolated compounds Stigmasterol (C1) & β – Sitosterol (C2) (H and E, 40 x). Micrographs showing normal (A) intact islets with cellular characteristics of pancreas; diabetic group (B) with degeneration of pancreatic acini, apathy of β cells (necrotic and fibrotic changes) and emaciated islets of Langerhans; EE 100 (C), EE 200 (D), C1 (E), C2 (F) and metformin (G) with refurbishment of normal cellular population of pancreatic acini/lobules and with size, shape of the islets. Abbreviations are as defined in Table 2.

254x190mm (96 x 96 DPI)