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ORIGINAL ARTICLE

Investigation of Antihyperglycaemic Activity of Banana (*Musa* sp. Var. Nanjangud rasa bale) Flower in Normal and Diabetic Rats

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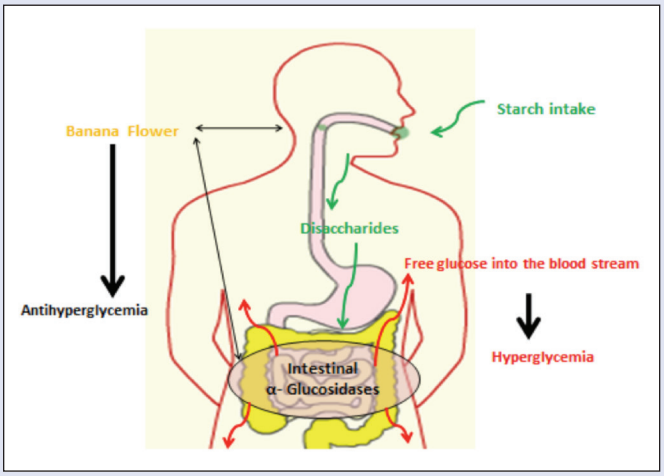
ABSTRACT

Background: The vital enzymes of starch digestion and absorption are intestinal α-glucosidases and their inhibition improves postprandial hyperglycaemia, constituting an effective mode of therapy in diabetes. **Objectives:** The present study was designed to assess the inhibitory potential of ethanol extract of banana flower (EF) on mammalian α-glucosidases and its pharmacological effects on postprandial hyperglycaemia in normal and alloxan-induced diabetic rats. **Materials and Methods:** EF was evaluated for its inhibitory potential and mode of inhibition on mammalian α-glucosidases. Further, the role of EF and its constituents Umbelliferone (C1) and Lupeol (C2) on glucose uptake using isolated rat hemi-diaphragm and insulinotropic activity using RINm5F (rat insulinoma) cell lines were determined. The phytocomponents in EF were also evaluated using GC-MS. **Results:** EF illustrated a dose-dependent inhibition for rat intestinal sucrase, maltase and *p*-nitrophenyl-α-D-glucopyranoside (pNPG) hydrolysis (IC_{50} values: 18.76±0.22, 25.54±0.10 and 76.42±1.12 µg/ml, respectively) and the mode of inhibition was non-competitive with low Ki values. Oral administration (100-200 mg/kg b.wt.) of EF significantly improved the maltose/glucose-induced postprandial hyperglycaemia in normal and alloxan-induced diabetic rats. EF, C1 and C2 exhibited stimulation of glucose uptake and a dose-dependent glucose-induced insulin secretion at both 4.5 and 16.7 mM glucose concentrations. Further, GC-MS analysis revealed significant levels of steroids (25.61%), diazoprogesterone (21.31%), sesquiterpene (11.78%) and other phytocomponents. **Conclusion:** EF inhibited α-glucosidases besides promoting glucose uptake and insulin secretion, resulting in antihyperglycaemic effect determining EF as a potent anti-diabetic agent.

Key words: Diabetes, ethanol extract, glucose uptake, α-glucosidase inhibition, postprandial hyperglycaemia, RINm5F cells



Abbreviations used: mg/dl: milligramsper deciliter, mM: millimolar, b.wt.: body weight.

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INTRODUCTION

Diabetes mellitus, a chronic metabolic defect is caused due to the collective effect of altered carbohydrate, protein, and lipid metabolism. The sedentary lifestyle and improper diet have accounted for the disease reaching an epidemic proportion over the past decades and on these lines, the International Diabetes Federation (IDF) predicts an average of 592 million diabetic patients by 2035.[1] Diabetes is characterized by persistent hyperglycaemia due to the defects in insulin secretion, insulin action, or both. Standard antihyperglycaemic drugs such as acarbose, miglitol, and voglibose mainly target the activity of α-glucosidase and α-amylase enzymes, which are the primary enzymes of the carbohydrate metabolism, responsible in converting complex carbohydrates into smaller sugar units.[2,3] While hydrolysis of starch into smaller oligosaccharides is catalyzed by pancreatic α-amylase, its further breakdown to free glucose units is carried out by intestinal α-glucosidases and inhibiting these enzymes renders an overall smooth glucose profile.[4]

Diabetes is also well characterized by peripheral insulin resistance, which in turn results in reduced glucose uptake by the tissues, leading to persistently high postprandial blood glucose levels.[5] Glucose uptake is promoted in the peripheral tissues by the administration of biguanide such as metformin.Owing to the adverse effects exerted by these synthetic therapeutic agents, their administration is less advocated.[6] In

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this regard, herbal formulations are identified as safer alternatives for anti-diabetic therapy and on the basis of their traditional use; several plants have been screened for their potential in reducing the postprandial hyperglycaemia and the induced diabetic complications.[7]

Banana is a popular tropical fruit relished world over. The secondary parts *viz.,* flower, pseudostem and rhizomeare used as formulations in several traditional medicines such as Ayurveda, to cure various ailments.[8] In our previous study, ethanol extract of banana flower (EF) with significant amount of Umbelliferone (C1) and Lupeol (C2) exhibited a remarkable antihyperglycaemic effect.[9] However, mechanism responsible for its anti-diabetic activity has not been defined.Hence, the present investigation was designed to (i) characterize the inhibition mode and kinetics of the EF on mammalian intestinalα-glucosidases; (ii) determine whether EF can suppress postprandial blood glucose *in vivo*; (iii) identify compounds that are involved in the anti-diabetic activity using GC-MS studies; (iv) assess their role on glucose uptake by isolated rat hemi-diaphragm and glucose-dependent insulinotropic activity in RINm5F (rat insulinoma) cell lines.

MATERIALS AND METHODS

Chemicals

Reagents and solvents used for extraction were procured from Merck (Mumbai, India). The standard drugs were purchased from Sigma-Aldrich Chemical Co (St. Louis, MO, USA). All other reagents were of analytical grade.

Plant material

Fresh inflorescences (*Musa* sp. cv. Nanjangud rasa bale) were garnered from banana cultivating farms of Nanjangud, Karnataka, India and authenticated with the assistance of the Department of Horticulture, Government of Karnataka, Mysore, India. The spathe was removed to recover the flowers, which were cleaned, chopped into pieces and dried (40°C) in an oven. The dried pieces were powdered using a laboratory homogenizer and stored at 4°C till use.

Preparation of extract and isolation of active compounds

The coarse powder was subjected to hot ethanol extraction using a Soxhlet apparatus (twice using 95% ethanol) and filtered. The filtrate was further concentrated *in vacuo* using rotary evaporator (Rotavapor R-200, Buchi, Switzerland). The active compounds present in the ethanol extract of banana flower (EF) were identified as Umbelliferone (C1) and Lupeol (C2) using various spectroscopic methods via successive solvent extraction followed by repeated silica gel column chromatography.[9]

GC-MS analysis

EF was subjected to gas chromatography–mass spectrometry (GC-MS) as described by Ramith*et al.*[3] to identify the phytochemical compounds. The structural identification of the compounds present in EF was performed by comparing known compounds present in the database library (National institute of standard and technology (NIST), Wiley8 and FAME database library).[3]

Isolation of α-glucosidase from rat small intestine

The small intestine of male Wistar albino rats (220 g) was detached after sacrificing them under ether anaesthesia. The crude α-glucosidase enzyme was isolated from small intestine according to the procedure described by Ramith *et al.*[3] The protein content was assessed and all the successive steps were performed at 4°C.[10]

Rat intestinal α-glucosidases inhibition assay

Rat intestinal α-glucosidases inhibitory potential of EF was determined by the method of Matsui *et al.*[11] with minor modifications. In brief, diverse concentrations of EF or acarbose were incubated with crude α-glucosidase enzyme at 37°C for 10 min. Further, the reaction was initiated by the addition of substrates maltose (5 mM), sucrose (50 mM) and p-nitrophenyl-α-D-glucopyranoside (pNPG, 0.50 mM) in phosphate buffer (50 mM, pH 6.8) to attain at a final reaction volume of 1000 µl and incubated at 37°C for 45, 30 and 20 min, respectively.[3] The termination of the reaction was then carried out by the addition of 1000 µl Tris base (0.5 M) and the inhibitory activity was assessed by measuring the absorbance of the liberated *p*-nitro phenol from pNPG at 405 nm, while the absorbance of the liberated glucose from sucrose/maltose was monitored by glucose oxidase method.[12] The experiments were performed in three individual sets and the enzyme inhibition data were expressed as IC_{50} value (the half maximal inhibitory concentration).

Kinetics of α-glucosidases enzyme inhibition

The α-glucosidases inhibition kinetics was evaluated with the increasing concentrations of maltose, sucrose and pNPG substrates versus the presence/absence (control) of three diverse concentrations (IC_{20}, IC_{40} and IC_{60}) of EF. The type of inhibition (i.e. competitive, non-competitive and uncompetitive or mixed-type), K_m (dissociation constant) and V_{max} (maximum reaction velocity) were determined by double reciprocal plot[13] of the substrate concentration and the velocity (1/V versus 1/[pNPG]). Further, the inhibitory constant (Ki) was determined by Dixon plot.[14]

Hypoglycaemic activity of EF in normal rats

Healthy Wistar rats of either sex (180-220 g) were procured from the animal house of JSS College of Pharmacy, Ootacamund, India. Prior to the experiments, the study was approved by Institutional Animal Ethics Committee (No.: JSSCP/IAEC/Ph.D./PH.COLOGY/01/2013-14) and conducted as per the guidelines of CPCSEA, Chennai, India. Throughout the course of the experiment, the animals were maintained in large hygienic cages at 25 ±1°C with a 12 ± 1 h day and night schedule. The rats were fed using standard rat feed and water *ad libitum*. The *in vivo* cytotoxicity studies were performed for EF in our previous study[15] and the doses were selected as per the OECD guidelines.[16]

The overnight fasted rats with fasting blood glucose (FBG) between 60 to 90 mg/dl were divided into eight groups (6 animals in each group). Animals in the experimental group were orally fed (by gauge needle in a final volume of 1ml) with 100 and 200 mg/kg body wt. of EF and 50 mg/kg body wt. of acarbose (maltose tolerance test) or 250 mg/kg body wt. of metformin (glucose tolerance test) dissolved in distilled water. Subsequently, 15 min post oral administration of EF, maltose or glucose (3 g/kg body wt.) was orally fed and the plasma glucose profile of individual rat was evaluated by glucose oxidase[12] method from the blood samples collected at 0, 30, 60 and 120 min (from the retro orbital plexus).[3]

Hypoglycaemic activity of EF in diabetic rats

Overnight fasted rats were rendered diabetic by intra peritoneal injection of 120 mg/kg alloxan monohydrate dissolved in freshly prepared saline. Animals exhibiting marked hyperglycaemia (FBG >200 mg/dl) 72 h post injection of alloxan, were considered as diabetic and were divided into four groups of six animals each. They were treated entirely similar to the maltose loaded normal rats, as explained earlier in the previous section.

Table 1: : Chemical profile of the ethanol extract of flower by GC-MS

Compounds detected[a]	Molecular formula	Molecular weight	Total % composition	Compound nature	Activity[b]
Undecanoic acid, ethyl ester	$C_{13}H_{26}O_2$	214	1.87	Ester	**
Phytol	$C_{20}H_{40}O$	296	4.30	Diterpene	Antimicrobial, diuretic, antiinflammatory and anticancer
1-Iodo-2-methylnonane	$C_{10}H_{21}I$	268	1.87	Iodine	Antimicrobial
1-Iodo-2-methylundecane	$C_{12}H_{25}I$	296	4.11	Iodine	Antimicrobial
Heptadecane, 2,6-dimethyl -	$C_{19}H_{40}$	268	5.05	Alkane	**
6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z) -	$C_{25}H_{36}O_2$	368	8.22	Ester	Antiinflammatory, hypocholesterol, hepatoprotective and anticancer
Diazoprogesterone	$C_{21}H_{30}N_4$	338	21.31	Female hormone	To control menstruation
Spiro [androst-5-ene-17, 1-cyclobutan]-21-one, 3-hydroxy-(3a,17a) -	$C_{22}H_{32}O_2$	328	25.61	Steroid	Antimicrobial, anticancer, antiinflammatory, diuretic antiasthma and antiarthritic
1,6,10-Dodecatrein-3-ol,3,7,11-trimethyl-, [S-(Z)] -	$C_{15}H_{26}O$	222	11.78	Sesquiterpene alcohol	Antimicrobial, antiinflammatory and antihyperlipidemic
1, 3-Bis-(2-cyclopropyl, 2-methyl cyclopropyl) but-2-en-1-one	$C_{18}H_{26}O$	258	15.89	Ketone	**

a. Compounds were identified by referring to NIST08s, WILEY8 and FAME libraries b. Activities were acknowledged by Dr Duke's Phytochemical and Ethnobotanical databases **. Activity not reported

Maintenance and usage of RINm5F cell lines

The RINm5F rat insulinoma cell lines were procured from National Centre for Cell Science (NCCS), Pune, India and 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_2 at 37°C. The optimization of the cell concentration and the insulin secreting assay was carried out as described by Rajesh *et al.*[17] All the 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 10^6 cells in 96-well microtitre plate and allowed to adhere for 24 h.The cells were washed once with sterile phosphate-buffered saline (PBS, pH 7.2) prior to 45 minincubation with fresh Krebs-Ringer bicarbonate HEPES (KRBH) buffer comprising of KH_2PO_4 (1.2 mM), $NaHCO_3$ (10 mM), NaCl (115 mM), KCl (4.7 mM), $CaCl_2$ (1.28 mM), MgSO4 (4 in lower case like in CaCl2) 4.7 H_2O (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 EF and its isolated compounds (C1 and C2). 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.[17]

In vitro glucose uptake assay

Glucose uptake by rat hemi-diaphragm was determined according to the method described by Prashantha *et al.*[18] with slight modifications. After sacrificing male Wistar albino rats (220 g) under ether anaesthesia, the diaphragms were detached expeditiously with minimal trauma and divided into two equal halves. The resulting hemi-diaphragms were rinsed with cold glucose free Tyrode solution to wash the blood clots and swiftly transferred to the six well microtitre plates (5 ml capacity, n=3). Furthermore, these were grouped as follows, Group 1 (control): 2 ml of Tyrode solution with 2% glucose; Group 2 (insulin treated): 2 ml of Tyrode solution with 2% glucose + 100 µl of regular insulin (0.4 U/ml, Biocon Ltd.,); Group 3 to 8: 2 ml of Tyrode solution with 2% glucose + 50 and 100 µg/ml of EF, C1 and C2; Group 9 to 14: 2 ml of Tyrode solution with 2% glucose + 50 and 100 µg/ml of EF, C1 and C2 + 100 µl of regular insulin. The initial glucose concentration was estimated by glucose oxidase method[12] by addition of distilled water with the final volume of 4 ml. The plates were further incubated at 37°C for 30 minutes with continuous shaking at 60 cycles per minute. Following the incubation, glucose uptake per gram of tissue was estimated as the difference between the initial and final glucose content in the incubated wells.

Statistical analysis

The experiments were done in triplicates. Results were expressed as Mean ± SE. Graph pad PRISM software (version 4.03, Inc., USA) was used for calculating IC_{50} values. The quantitative glucose tolerance of each animal was determined by the area under curve (AUC) method. Statistical comparisons between normal and 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 or less.

RESULTS

GC-MS profile study of the active EF

GC-MS analysis of EF revealed about ten active compounds with highest concentrations [Table 1]. GC-MS analysis indicated the presence of various active components, including steroids (25.61%), sesquiterpenes (11.78%), ketones (15.89%), esters (10.09%), alkanes (5.05%), iodine compounds (5.98%) and female hormone (21.31%). Among the compounds identified, the most prevailing compounds were the Spiro [androst-5-ene-17, 1-cyclobutan]-21-one, 3-hydroxy-(3a, 17a), followed by diazoprogesterone and 1, 6, 10-Dodecatrein-3-ol, 3, 7, 11-trimethyl-, [S-(Z)].

Kinetic analysis of α-glucosidase inhibition by EF

EF exerted a concentration dependent inhibition on rat intestinal sucrase, maltase and pNPG hydrolysis as shown in Figure 1a. The inhibitory activity of EF (IC_{50}: 18.76±0.22, 25.54±0.10 and 76.42±1.12 µg/ml

respectively, for substrates sucrose, maltose and pNPG respectively) was higher than that of the therapeutic drug acarbose (42.12±0.69, 114.4±3.68 and 161.60±6.34 µg/ml). Kinetics of the enzyme inhibition was evaluated by incubating the discrete concentrations of substrates *viz.*, sucrose, maltose and pNPG in the absence (control) or presence of IC_{20}, IC_{40} and IC_{60} inhibitory concentrations of EF. [Km], [Vmax] values and the mode of inhibition were determined graphically using Lineweaver Burk plots. The plots revealed that the intersecting point for different concentrations of sucrose [Figure 1b], maltose [Figure 1c] and pNPG [Figure 1d] arise from the same x-intercept as the uninhibited enzyme. With the increasing concentrations of EF, both the slope and the vertical axis intercept increased, whereas, the horizontal axis intercept (-1/Km) remained the same. The kinetic results revealed that EF retarded the maximum velocity (Vmax) of the reaction (with increasing concentrations) catalyzed by α-glucosidases without much change in Km values. These results indicated that the mechanism of α-glucosidase inhibition was reversible, corresponding to the classical pattern of non-competitive inhibition.[9] Besides, enzyme kinetic derivations using the Dixon plot calculations established that the inhibition constant (Ki) of EF was calculated to be 10.52±0.11, 34.43±0.07 and 119.79±0.10 µg for sucrase, maltase and pNPG hydrolysis, respectively [Table 2].

Antihyperglycaemic effect of EF in maltose-loaded normal rats

The promising inhibitory potential of EF on disaccharidases and pNPG hydrolysis led us to examine the inhibition of EF on the postprandial glucose concentration in normal and diabetic rats loaded with high-maltose diet. As shown in Figure 2a, upsurge in postprandial plasma glucose level was ameliorated by EF after the administration of high maltose diet and changed from 73 to 128 mg/dl within 30 minutes. While in the control group, postprandial plasma glucose level increased from 77 to 202 mg/dl in 30 minutes after the administration of maltose diet and declined subsequently. On the contrary, the control group exhibited a reduction in the glycaemic response by 22.14% ($p < 0.05$) and 30.70% ($p < 0.05$) upon EF treatment at 100 and 200 mg/kg b.wt., respectively and 11.99% ($p < 0.05$) on acarbose treatment.[3]

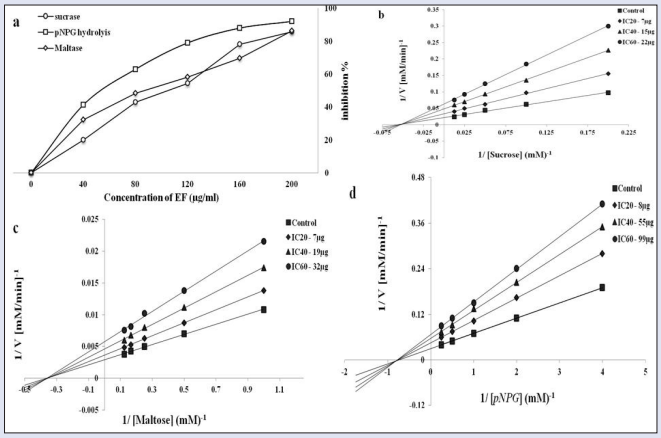

Figure 1: Inhibitory effect by ethanol extract of flower (EF) on rat intestinal sucrase, maltase and pNPG hydrolysis (a). Lineweaver-Burk plot of substrate dependent enzyme kinetics on inhibition of rat intestinal sucrase (b), maltase (c) and pNPG hydrolysis (d) activity by EF. α-glucosidase was incubated with different concentrations of sucrose (5-80 M), maltose (1-8 mM) and p-Nitrophenyl-α-D-glucopyranoside (pNPG, 0.25-4 mM) in the absence (control) or presence of EF at IC_{20}, IC_{40} and IC_{60} inhibiting concentrations (µg /ml).

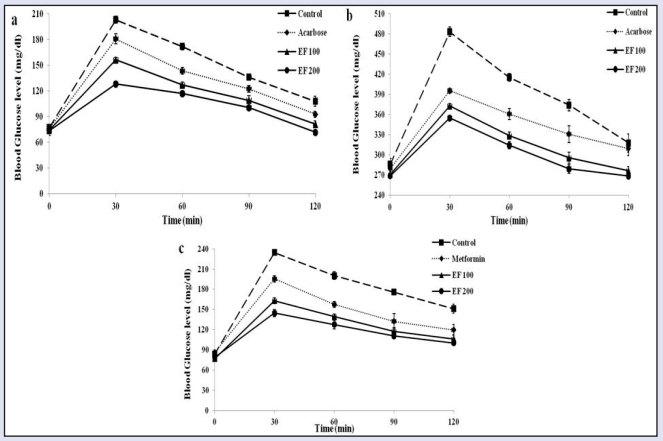

Figure 2: : Inhibitory effects of ethanol extract of flower (EF) on postprandial plasma glucose after maltose/glucose loading in normal and diabetic rats. Glycaemic response curve in normal rats (a) and diabetic rats (b) after maltose challenge; glycaemic response curve in normal rats after glucose challenge (c). The normal and diabetic rats fasted for 18 h were received maltose/glucose (3g/kg body wt.) and EF at two different doses (100 and 200 mg/kg body wt.). Control animals were given only maltose/glucose plus 50 mg/kg body wt. of acarbose (positive control). Plasma glucose levels were monitored at 0 (fasting), 30, 60, 90 and 120 min. Data are expressed as the mean± SE, n = 6.

Table 2: : Enzyme kinetics of rat intestinal α-glucosidase by ethanol extract of flower (EF)

Substrates	Inhibitor	Mode of inhibition[x]	Km (mM)	Vmax (10^3(µM/min)$^{-1}$	K_i(µg)[y]	IC_{50}[z](µg/ml)
Sucrose	Control	Non competitive	18.87	46.95	10.52±0.11	18.76±0.22
	EE 9 µg		18.62	30.30		
	EE 15 µg		18.87	20.83		
	EE 22 µg		18.93	15.85		
Maltose	Control	Non competitive	2.76	344.83	34.43±0.07	25.54±0.10
	EE 7 µg		2.76	270.27		
	EE 19 µg		2.81	217.39		
	EE 32 µg		2.74	172.41		
*p*NPG	Control	Non competitive	1.33	33.33	119.79±0.10	76.42±1.12
	EE 8 µg		1.30	22.17		
	EE 55 µg		1.29	17.57		
	EE 99 µg		1.28	14.88		

x inhibition mode was determined from Line weaver and Burk plot. y Ki, the inhibitory constant values were determined from the Dixon plots. Values are mean ± SE (n = 3). z Values are expressed as mean ± SE. The IC 50 value is defined as the inhibitor concentration to inhibit 50% of enzyme activity under assay conditions.

Antihyperglycaemic effect of EF in maltose-loaded diabetic rats

Figure 2b illustrates the postprandial plasma glucose profile of maltose loaded diabetic rats at different time intervals, treated with two concentrations of EF (100 and 200 mg/kg b.wt.) and acarbose (50 mg/kg b.wt.). The increased postprandial plasma glucose levels in response to maltose treatment in diabetic rats were significantly impeded by EF treatment in a dose dependent manner as against the control and acarbose treated rats. The increasing $AUC_{0\text{-}120min}$ (area under the glycaemic curve) as a result of maltose load in the diabetic rats was significantly dropped owing to EF treatment with both doses by 19.29% ($p < 0.05$) and 22.71% ($p < 0.05$), respectively. On the contrary, the acarbose treatment reduced $AUC_{0\text{-}120min}$ similar to our previous studies.[3]

Antihyperglycaemic effect of EF in glucose-loaded normal rats

The *in vitro* and *in vivo* studies explained that EF ameliorated postprandial hyperglycaemia by the inhibition of α-glucosidases in the small intestine of normal and diabetic rats. Thus, to investigate its inhibitory effect on the glucose uptake in the small intestine, postprandial plasma glucose level was measured after glucose load to normal rats treated with EF and metformin. [Figure 2c] illustrates the effect of EF on postprandial plasma glucose profile in normal rats after an oral glucose diet. Upsurge in plasma glucose concentration after 30 min of glucose diet is considerably ameliorated in the EF treated rats, compared to control group of rats suggesting its influence on the glucose absorption as well. In comparison to the control group, the whole glycaemic response was reduced by 29.78% ($p < 0.05$) and 35.12% ($p < 0.05$) upon EF treatment at both the doses, whereas metformin reduced the incremental $AUC_{0\text{-}120min}$ similar to our previous studies.[3]

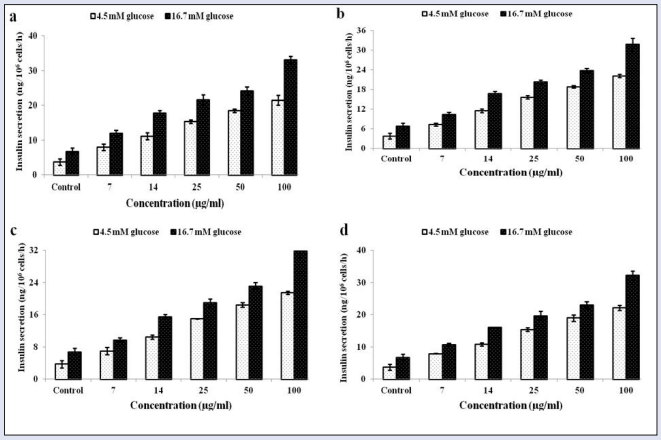

Figure 3: : The effect of diverse concentrations of (a) ethanol extract of flower, (b) Umbelliferone and (c) Lupeol (compounds isolated from EF) and (d) tolbutamide (positive control) on insulin secretion in RINm5F cells in the presence of 4.5 and 16.7 mM glucose load. Data are expressed as the mean± SE, n = 3 of independent experiments.

In vitro Insulin secretion in RINm5F cells

The effects of EF and its isolated compounds on insulin secretion (in RINm5F cells) in 4.5 and 16.7 mM glucose concentrations, which would represent normal and diabetic conditions respectively, is given in Figure 3a-d. A dose dependent increase in insulin secretion was observed at both 4.5 and 16.7 mM glucose after 60 minutes treatment with diverse concentrations of EF [Figure 3a], Umbelliferone [Figure 3b] and Lupeol [Figure 3c] when compared with respective control and tolbutamide [Figure 3d] treated group.

In vitro glucose utilization by isolated hemi-diaphragm of rats

EF and its constituents exhibited concentration dependent utilization of glucose uptake as shown in Table 3. The stimulation of glucose uptake by the diaphragm both in presence and absence of insulinafter 30 minutes revealed that, EF, C1 and C2 (50 and 100 µg/ml, respectively) exhibited a significantly ($p < 0.05$) higher activity compared to control groups. It seemed that EF and its constituents have a direct peripheral glucose uptake stimulatory action.

DISCUSSION

Diabetes is intricately associated with several microvascular and cardiovascular complications, which are caused due to persistent hyperglycaemic state. Impaired insulin and glucagon secretion along with the abnormalities in the glucose uptake in liver and peripheral tissues, as well as the hepatic glucose production contributes greatly to the elevation in the plasma glucose levels in diabetic patients.[19] One of the prime means of inducing antihyperglycaemia is by inhibiting the key enzyme, α-glucosidases, which catalyzes the final step of carbohydrate metabolism.[4] This enzyme located in the brush-border surface of the intestinal cells greatly contributes to the postprandial plasma glucose concentration, thereby inducing a hyperglycaemic state. A potent antihyperglycaemic agent inhibits the activity of α-glucosidases thereby prolonging the duration of carbohydrate absorption and lowering plasma glucose levels.[2] In the present study, we have evaluated the inhibitory potential of EF against the activities of maltase, sucrase and pNPG hydrolysis of rat intestinal epithelium. The results suggest a dose dependent inhibition exerted by EF at microgram concentrations, with the efficiency better than the standard drug acarbose.The nature of inhibition as inferred by the kinetics demonstrated a reversible, non-

Table 3: Evaluation of glucose uptake for ethanol extract of flower and its isolated compounds by isolated rat hemi-diaphragm at two different doses with the presence/absence of insulin

	Glucose uptake * (mg/g/30 min)			
	No insulin		With insulin	
	50µg/ml	100µg/ml	50µg/ml	100µg/ml
Control	17.39±0.75^{a}	17.39±0.75^{a}	25.72±0.87^{a}	25.72±0.87^{a}
EF	25.29±0.75^{c}	31.43±1.48^{c}	34.26±0.93^{c}	41.72±1.69^{b}
Umbelliferone	21.12±1.00^{b}	24.98±1.79^{b}	31.42±0.86^{b}	39.55±0.51^{b}
Lupeol	20.42±0.58^{b}	24.27±0.92^{b}	30.38±0.78^{b}	39.04±2.13^{b}
Rosiglitazone$^{\#}$	30.76±0.67^{d}	34.26±1.27^{d}	41.71±0.48^{d}	46.38±0.98^{c}

*Values are expressed as mean ± SE. Means in the same column with distinct superscripts are significantly different ($p \leq 0.05$) as separated by Duncan multiple range test $^{\#}$Rosiglitazone was used as a positive control

competitive mode of inhibition for sucrase, maltase and pNPG hydrolysis with lower Ki values. From the studies, it can be substantiated that EF binds to a site other than the active site of the enzyme, thereby retarding the enzyme substrate reaction, without competing with the substrate for the active site of the enzyme. Likewise, the mode of inhibition being reversible, the stability of the enzyme is also not affected by its inhibition. This is advantageous over the irreversible type since, it would then lead to hypoglycaemic state due to a permanent carbohydrate malabsorption.

The *in vitro* studies provided a basis for further *in vivo* studies to evaluate the effects of EF on postprandial hyperglycaemia associated with carbohydrate challenge in normal and diabetic rat models. In normal rats, the effect of EF was evaluated after monosaccharide and disaccharide load, by administering glucose and maltose, respectively. The postprandial plasma glucose levels (of rats fed with 3 g/kg b.wt. of maltose and glucose) were considerably reduced upon administration of EF (100 and 200 mg/kg of b.wt. doses), with the concentration of 200 mg/kg b.wt. fairing better than 100 mg/kg b.wt. treatment. However, the results were better in comparison with the standard drug acarbose in augmenting the glycaemic response. Antihyperglycaemic effects exhibited by EF on both maltose and glucose loaded rats suggest the effective inhibition by EF on both α-glucosidases (by amelioration of maltose load) and glucose absorption (by amelioration of glucose load). Subsequently, the role of EF on amelioration of hyperglycaemia on maltose-challenged alloxan-induced diabetic rats was assessed and the results were promising as a dose-dependent reduction of plasma glucose levels were witnessed in EF treated diabetic rats. The results from the *in vitro* and the *in vivo* studies suggest that the antihyperglycaemic activity by EF is positively associated with the α-glucosidase inhibition, which limits the circulating levels of plasma glucose.

The major circulating glucose is utilized by the peripheral tissues by means of glucose uptake. The prime site for glucose uptake and insulin action is the skeletal muscle, which represents about 30-40% of the total body weight.[20] In our study, we have evaluated the glucose uptake using rat hemi-diaphragm, which is a reliable representative of the peripheral glucose uptake by the skeletal muscle cells.[21] The findings confirm that EF and its constituents C1 and C2 possess insulin-like properties as implied by the enhancement in the glucose uptake after its treatment and thus suggest that EF, C1 and C2 have some peripheral role either alone or together with insulin, which promotes glucose uptake in the muscle cells.

Considering the primary role of insulin in peripheral glucose uptake, it can also be anticipated that EF and its constituents might have an effect in the insulin secretion and to assess this, *in vitro* insulin secretion study using rat insulinoma cells (RINm5F) was carried out. The *in vitro* study illustrated an insulinotropic activity by enhanced glucose-induced insulin secretion after treatment with EF and its constituents at both 4.5 and 16.7 mM glucose concentrations. The study reveals that EF exerts a collective effect (along with inhibiting α-glucosidases, it also promotes glucose uptake and insulin secretion) in extending antihyperglycaemic effects, which may be due to the enormous bioactive principles present in it.

Our study also confirmed the existence of various phytocomponents with different chemical structures and using Dr. Duke's phytochemical and ethnobotanical database (online), the biological activity of the identified bioactive compounds were ascertained. Our previous study also reports the isolation and characterization of the bioactive components, Umbelliferone and Lupeol present in significant amounts, which were proven to be potential α-glucosidase inhibitors.[9] Considering these reports, it can be anticipated that the positive anti-diabetic activity exhibited by EF is due to the synergistic action of all these compounds.

CONCLUSION

In summary, the cumulative effect exerted by EF on inhibition of α-glucosidase, enhancement of glucose utilization and inhibition of glucose absorption have resulted in the suppression of the maltose/glucose-induced postprandial hyperglycaemia in normal and diabetic rats, which implies its potential as anti-diabetic agent. The antihyperglycaemic effects observed by EF may be attributed to the enumerable bioactive principles, which derive a huge attention in further characterization of these components. Thus, the findings of the present study are noteworthy in designing potential drug attributes as well as novel dietary resources from banana flower for diabetes management.

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Conflicts of interest

There are no conflicts of interest

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