Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
23(4): 706-715, Jul./Aug. 2013

Beneficial effects of banana leaves (*Musa* x *paradisiaca*) on glucose homeostasis: multiple sites of action

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Article

Received 29 Apr 2013
Accepted 4 Aug 2013
Available online 13 Sep 2013

Keywords:
banana
glycation
glycemia
HPLC
insulin
Musa x *paradisiaca*

Abstract: The acute effect of crude extract, *n*-butanol and aqueous residual fractions of *Musa* x *paradisiaca* L., Musaceae, leaves on glycemia, serum insulin secretion and glycogen content in an *in vivo* approach was evaluated. In addition, the *in vitro* effect on disaccharidases activity and albumin glycation was studied. The crude extract and fractions, *n*-butanol and aqueous residual, reduced glycemia and increased liver glycogen content in hyperglycemic rats, inhibited maltase activity and the formation of advanced glycation end-products *in vitro*. Also, a significant increase in insulin secretion and muscle glycogen content in hyperglycemic rats was observed with oral administration of the *n*-butanol fraction. Phytochemical analysis demonstrated the presence of rutin in crude extract and fractions of *M.* x *paradisiaca* leaves as the major compound. These beneficial effects on the regulation of glucose homeostasis observed for *M.* x *paradisiaca* leaves and the presence of rutin as the major compound indicate potential anti-diabetic properties, since previous studies have been reported that rutin can modulate glucose homeostasis.

ISSN 0102-695X
DOI: 10.1590/S0102-695X2013005000062

Introduction

Diabetes mellitus is the most common metabolic disorder and is a major cause of ill health all over the world. It is caused by defects in insulin secretion or action and, consequently, is characterized by hyperglycemia. Several pathogenic processes are involved in the development of diabetes and the chronic hyperglycemia is associated with long-term damage, dysfunction and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Treatment of hyperglycemia in diabetes involves diet control, exercise and the use of oral anti-diabetic drugs, insulin therapy or combination of both (American Diabetes Association, 2011). Nonetheless, they fail to alter the course of complications and tend to result in undesirable side effects.

Plants continue to be an important source of bioactive compounds and involve a multidisciplinary approach combining ethnobotanical, phytochemical and biological techniques to provide new chemical compounds. For many years people have used plants to treat diabetes. In this context, the hypoglycemic effect of several plants used as antidiabetic remedies has been confirmed, and the mechanisms of hypoglycemic activity of these plants and their major compounds are being investigated (Jung et al., 2006).

Musa x *paradisiaca* L., Musaceae, popularly known as 'banana', is a perennial tree-like herb cultivated in many tropical and subtropical regions around the world. Banana, eaten as a fruit or a vegetable, is one of the most important crops in several countries due to its enriched food and versatile medicinal value. Various parts of the *Musa* plants have been used orally or topically as remedies in folk medicine and some studies have demonstrated this medicinal potential. The fruits, peel, leaves, roots and pseudostem of *Musa* plants have shown antiulcerogenic, antioxidant and antimicrobial activity, among others activities (Pannangpetch et al., 2001; Eleazu

et al., 2010; Karadi et al., 2011). In addition, studies have shown that some species of *Musa* possess antidiabetic, antihyperglycemic and hypoglycemic activity (Ojewole & Adewunmi, 2003; Mallick et al., 2006; Adewoye et al., 2009).

The presence of bioactive compounds like apigenin glycosides, myricetin glycoside, myricetin-3-*O*-rutinoside, naringenin glycosides, kaempferol-3-*O*-rutinoside, dopamine, *N*-acetyl serotonin, and rutin, has been reported in different species of *Musa* (Pothavorn et al., 2010). However, as far as we aware, there is no reports concerning chemical characterization and pharmacological properties of their leaves. Thus, the aim of the present study was to investigate the *in vivo* and *in vitro* effect of crude extract, *n*-butanol and aqueous residual fractions of leaves of *Musa* x *paradisiaca* on serum glucose levels, insulin secretion, liver and muscle glycogen content, serum albumin glycation and intestinal disaccharidase activity. Also, a phytochemical characterization of crude extract and fractions was carried out.

Material and Methods

Chemicals

Rutin (≥98%), glycogen, bovine serum albumin (BSA), tolbutamide were purchased from Sigma Chemical Company® (St. Louis, MO, USA). Glucose, fructose, maltose, sucrose and all other solvents were purchased from Vetec® AG (Rio de Janeiro, Brazil). All reagents were of analytical grade. The solvents used for HPLC analysis were purchased from Tedia® (HPLC grade; Fairfield, OH, USA). Enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of rat insulin (catalogue no. EZRMI-13K) was purchased from Millipore (St Charles, MO, USA).

Plant material

Leaves of *Musa* x *paradisiaca* L., Musaceae, were collected in Florianópolis, State of Santa Catarina, Brazil, Fazenda Experimental da Ressacada (27º 41' 06.28" S; 48º32' 38.81" O) in April 2008. The voucher specimen was identified by Dr. Geraldo Ceni Coelho and is deposited in the herbarium at Universidade Federal de Santa Catarina (FLOR 3832).

Preparation of the extracts and fractions of Musa x paradisiaca

The crude extract (CE) and fractions were prepared according to Costa et al. (2011) with minor modifications. Briefly, dried leaves (50 g) of *M.* x *paradisiaca* were crushed and extracted under reflux (90 ºC) with 500 mL of ethanol 40% (v/v) for 30 min. After cooling, the extract was filtered, the volume was adjusted to 500 mL with water, and the extract was separated into two fractions of 250 mL. One fraction was evaporated under reduced pressure to dryness to obtain CE. The ethanol content of the second fraction was removed under reduced pressure, its volume was adjusted to 250 mL with water, and this aqueous suspension was partitioned (3 x 100 mL) with *n*-BuOH, yielding the *n*-BuOH (BF) and aqueous residual fractions (ARF).

Chemical characterization of crude extract and fractions

The presence of different constituents in crude extract and fractions from *M.* x *paradisiaca* was established by thin-layer chromatography (TLC) on silica gel plates (Merck 60 F_{254} 20x20 cm) using several mobile phases. Detection was performed, respectively, with chlorosulfonic acid-glacial acetic acid reagent spraying and heating for terpenoids, and for phenolic compounds, fluorescence at 365 nm after spraying with 1% diphenylboryloxyethylamine in MeOH. The high performance liquid chromatography (HPLC) analyses were performed in a PerkinElmer Series 200 HPLC, composed of a Photo Diode Array Detector (PDA), quaternary pump and autosampler. The data acquisition system was TotalChrom Workstation software. All samples were dissolved in MeOH:H_2O (1:1 v/v), filtered using a 0.45 µm syringe filter (PVDF, Millipore®) and 10 µL aliquots were injected for HPLC analysis. The extracts and fractions were analyzed at 1,000 mg/mL while the rutin standard solution was analyzed at 100 µg/mL. The separation was performed on a Perkin Elmer Brownlee Choice C_{18} column (250 x 4.6 mm i.d.; 5µm) and the mobile phase used was a gradient of solvent A (acetonitrile) and solvent B (acetic acid 1%, adjusted to pH 3.0) as follow: 10-20% A (0-40 min) and isocratic 20% A (40-45 min). The flow rate was kept at 1.0 mL/min. The chromatograms were recorded at 340 nm while the UV spectra were monitored over the range of 200-450 nm. The flavonoids in the CE, BF and ARF of *M.* x *paradisiaca* were characterized by comparing the retention time and UV spectra with the reference standards, and by the co-injection of the sample and authentic samples (Costa et al., 2011).

In vivo *assays*

Animals

The male Wistar rats (190-220 g) used in this study were bred in animal facility and housed in an air-conditioned room (approximately 22 ºC) with controlled lighting on a 12:12 h light/dark cycle (lights on from 6 to 18 h). The animals were maintained with pelleted food

(Nuvital, Nuvilab CR1, Curitiba, PR, Brazil), while tap water was available *ad libitum*. Fasted rats were deprived of food for at least 16 h but allowed free access to water. All the animals were monitored and maintained in accordance with the ethical recommendations of the Brazilian Veterinary Medicine Council and the Brazilian College of Animal Experimentation. This study was approved by the Committee for Ethics in Animal Research of UFSC (Protocol CEUA PP00398).

Oral glucose tolerance curve (OGTC)

Fasted rats were divided into different groups of six animals for each treatment. Group I, hyperglycemic rats that received glucose (4 g/kg, 8.9 M); Group II, rats that received tolbutamide at a dose of 100 mg/kg; Group III, rats that received the CE at doses of 50, 100 and 200 mg/kg; Group IV, rats that received BF at doses of 50 and 100 mg/kg; Group V, rats that received ARF at doses of 50 and 100 mg/kg. The glycemia was measured before the rats received the treatment (zero time). The rats were fed with extract and fractions (dissolved in water) and after 30 min they were administered with glucose (4g/kg, 8.9 M) orally. The glycemia was measured at 15, 30, 60 and 180 min after administration of glucose. All treatments were administrated by oral gavage.

Determination of the serum glucose concentration

Blood samples were collected from the tail vein, centrifuged and the serum was used to determine the glycemia by glucose-oxidase colorimetric enzyme method according to the manufacturer's instructions (Gold Analisa® commercial kit).

Glycogen content measurements

The soleus muscle and liver were harvested from untreated hyperglycemic rats, treated with CE (200 mg/kg), BF (50 mg/kg) and ARF (100 mg/kg) and used for the assay of glycogen content immediately after 3 h of treatment. Glycogen was isolated from tissues as described by Krisman (1962). The tissues were weighed, homogenized in 33% KOH and boiled at 100 °C for 20 min, with occasional stirring. After cooling, 96% ethanol was added to the samples which were then heated to boiling followed by cooling in an ice bath to aid the precipitation of glycogen. The homogenates were centrifuged at 1300 x g for 15 min, the supernatant was discarded and the pellets were neutralized with saturated NH4Cl before being maintained at 100 °C for 5 min, washed and resolubilized in water. Glycogen content was determined by treatment with iodine reagent and the absorbance was measured at 460 nm. The results are expressed as mg of glycogen/g of tissue.

Insulin serum measurements

The insulin levels were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. The range of values detected by this assay was 0.2 ng/mL to 10 ng/mL. The intra- and inter-assay coefficients of variation (CV) for insulin were 3.22 and 6.95, respectively, with a sensitivity of 0.2 ng/mL. All insulin levels were estimated by means of colorimetric measurements at 450 nm with an ELISA plate reader (Organon Teknika, Roseland, NJ, USA) by interpolation from a standard curve. Samples were analyzed in duplicate and results were expressed as ng of insulin serum mL-1.

In vitro *assays*

Formation of advanced glycation end-products in the bovine serum albumin/glucose and fructose systems

Advanced glycation end-products (AGE) were formed in the *in vitro* system using a previously described method (Kiho et al., 2004). In brief, BSA (10 mg/mL) in phosphate buffered-saline (PBS, pH 7.4) containing 0.02% sodium azide was incubated with glucose (500 mM) or fructose (100 mM) at 37 °C for 14 and 28 days in the absence (control) and presence of the CE, BF or ARF of *M.* x *paradisiaca* (2.5 and 5.0 µg/mL). The protein, glucose or fructose, and the prospective inhibitor were simultaneously introduced into the incubation mixture. Each solution was kept in the dark in a capped vial, and incubation was allowed to proceed in triplicate vials. To the time-course experiments on AGE formation it was measured the characteristic fluorescence (excitation wavelength of 370 nm and emission wavelength of 440 nm) with Infiniti M200 (TECAN).

Disaccharidase extraction and assays

A segment of the small intestine was removed, washed in 0.9% NaCl solution, dried on filter paper, weighed, trimmed and homogenized (300 rpm) with 0.9% NaCl (400 mg of duodenum per mL) for 1 min at 4 ºC. The resulting homogenate was centrifuged at 8000 x g for 8 min and supernatant was collected. The supernatant was used for the measurement of *in vitro* maltase, sucrase and lactase activities and for total protein determination. Maltase (EC 3.2.1.20), lactase (EC 3.2.1.23) and sucrase (EC 3.2.1.48) activities were determined using a glucose diagnosis kit based on the glucose oxidase reagent. For determination of disaccharidase activity 50 µL of supernatant were pre-incubated at 37 °C for 5 min, in the absence (control) or in the presence of the CE, BF or ARF of *M.* x *paradisiaca* (treated groups). The concentrations used were 500, 1000 and 1500 µg/mL. The duodenum supernatant were then incubated at 37 °C for 5 min with 25 µL of the substrate

corresponding to 0.056 µM of maltose, sucrose or lactose (Pereira et al., 2011). One enzyme unit (U) was defined as the amount of enzyme that catalyzed the release of 1 µM of glucose per min under the assay conditions. The specific activity was defined as enzyme activity (U) per mg of protein. Protein concentration was determined by the method described by Lowry et al. (1951) method using bovine serum albumin (BSA) as the standard. The assays were performed in duplicate and conducted along with appropriate controls.

Data and statistical analysis

Data were expressed as mean±SEM. One-way analysis of variance (ANOVA) followed by the Bonferroni post-hoc test or unpaired Student's *t*-test to identify significant differences between groups using the GraphPad Prism Software (3.0 version). Differences were considered to be significant at $p \leq 0.05$.

Results

Phytochemical characterization

The chemical composition of CE and BF and ARF fractions of *Musa* x *paradisiaca* were determined by TLC and by RP-HPLC. The TLC analyses showed a predominance of flavonoids and the major spot detected in all analyses showed Rf and color similar to rutin (data not shown). Figure 1 shows the HPLC profiles for CE, BF and ARF of *M.* x *paradisiaca*. These analyzes identified the presence of rutin (rt=30.5 min) on crude extracts and fractions of *M.* x *paradisiaca* leaves as the major compound.

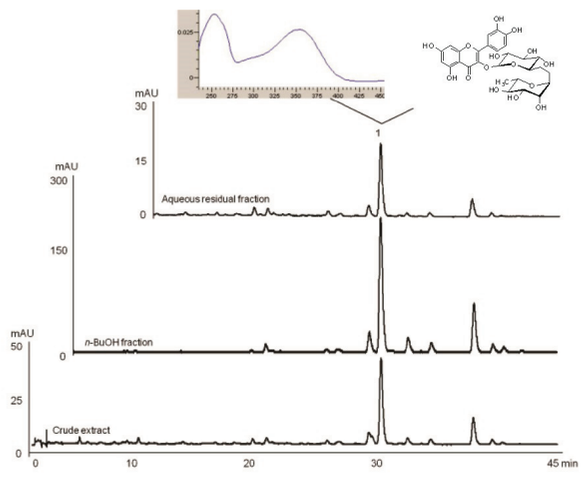

Figure 1. HPLC chromatograms of crude extract, aqueous residual fraction and *n*-butanol fraction of leaves of *M.* x *paradisiaca* with photo-diode array (PDA) detection at 340 nm. 1. rutin (rt 30.6 min).

Effect of crude extract and n-*BuOH and aqueous residual fractions of* M. *x* paradisiaca *on the oral glucose tolerance curve*

As expected, after starting the glucose tolerance test the serum glucose concentration was significantly increased when compared with zero time. Tolbutamide (100 mg/kg) an oral hypoglycemic agent of sulfonylurea class was used as a positive control and produced a typical serum glucose lowering at 15, 30 and 60 min compared to the hyperglycemic group (Table 1). The CE at all doses tested (50, 100 and 200 mg/kg) was effective in reducing the glycemia at different times after oral treatment when compared with the respective hyperglycemic control group (Table 1). The CE (200 mg/kg) produced the best antihyperglycemic profile from 15 to 60 min and the maximum reduction observed was 19% at 30 min, compared to tolbutamide. Table 1 also shows the effect of the BF at 50 and 100 mg/kg on oral glucose tolerance curve (OGTC). Although both doses showed an antihyperglycemic effect, the 50 mg/kg dose was more effective in terms of serum glucose lowering than 100 mg/kg, since it reduced significantly the glycemia at 15, 30 and 60 min. In addition, oral administration of ARF of also reduced the serum glucose levels in hyperglycemic rats at the two doses tested (50 and 100 mg/kg). The antihyperglycemic effect observed was around 22% and 25% at 30 and 60 min, respectively, after treatment with 100 mg/kg. At 180 min, glycemic levels were similar to the respective data for the hyperglycemic control groups.

On the other hand, acute treatments with CE, BF and ARF tested were ineffective to change serum glucose levels in alloxan-induced diabetic rats (data not shown).

Effect of crude extract and n-*BuOH and aqueous residual fractions on the glycogen content in soleus muscle and liver*

The glycogen content in the soleus muscle and liver of hyperglycemic normal rats after acute treatment with CE (200 mg/kg), BF (50 mg/kg) and ARF (100 mg/kg). Figure 2A shows that only BF were able to significantly increase the glycogen content in soleus muscle (183%) when compared with hyperglycemic control rats at 3 h after treatment.

Additionally, the CE, BF and ARF showed a stimulatory effect on hepatic glycogen storage (Figure 2B). When compared with hyperglycemic rats the CE and ARF significantly increased glycogen content in the liver around 123 and 136%, respectively. In this experimental condition, the maximum effect observed was around 142% for the BF.

Table 1. Acute effect of crude extract (CE), *n*-butanol fraction (BF) and aqueous residual fraction (ARF) of *M.* x *paradisiaca* on serum glucose levels (mg/dL) in oral glucose tolerance curve.

Time (min)	Group I Hyper Glucose (4 g/kg)	Group II Hyper+ tolbutamide (100 mg/kg)	Group III Hyper+ CE			Group IV Hyper + BF		Group V Hyper + ARF	
			50 mg/kg	100 mg/kg	200 mg/kg	50 mg/kg	100 mg/kg	50mg/kg	100 mg/kg
0	112±4	111±3	120±3	110±2	110±3	121±4	123±3	103±2	110±5
15	162±8	134±9**	137±8*	145±8	138±3*	128±6**	157±5	148±6	147±4
30	185±6	140±9***	151±5**	147±7**	150±5***	152±4**	155±4**	149±4**	144±4***
60	164±4	128±7***	171±8	139±8*	136±1*	141±6*	143±5	141±5*	123±5***
180	135±4	123±2	143±5	137±3	144±5	142±3	130±3	134±5	129±2

Values are expressed as mean±SEM; n=6 in duplicate for each treatment. Statistically significant difference compared to the corresponding hyperglycemic group. *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

Effect of crude extract and n-*BuOH and aqueous residual fractions on insulin secretion*

The insulin secretion after *in vivo* treatment with the extract and fractions of *M.* x *paradisiaca* was measured. Serum insulin levels were determined in fasted rats after an oral glucose loading (4 g/kg), as shown in Table 2. BF (50 mg/kg) stimulated significantly the insulin secretion at 15 min by around 154% when compared to the hyperglycemic control group (Table 2). So, the increase in glycogen content in the soleus muscle may be due to insulin release from β-cells stimulated by BF treatment. In addition, the high serum insulin secretion is according with the antihyperglycemic effect of the BF at a dose of 50 mg/kg seen on the glucose tolerance curve (Table 1).

Effect of crude extract and n-*BuOH and aqueous residual fractions on in vitro albumin glycation*

In the method adopted in this study, BSA was chosen as the model protein and glucose or fructose was used as the glycated agent. The BSA-reducing sugar system is an *in vitro* model widely used in non-enzymatic glycation studies. Proteins can be modified when exposed to reducing sugars through the spontaneous glycation process. The sugar-mediated fluorescence intensity, which is a characteristic of AGE, increases during incubation at 37 °C for a long period. In Figure 3A, B, C and D the fluorescence intensity of the products (AGE) formed in the BSA-glycation model can be observed. After incubation for both periods analyzed (14 and 28 days) it was clear that the formation of AGE was significantly increased in the BSA/glucose (Figure 3A and B) and BSA/fructose (Figure 3C and D) systems when compared to the basal control group.

Figure 3A shows the *in vitro* ability of CE and BF to suppress the AGE formation in the BSA/glucose system after fourteen days of incubation. All concentrations of CE, BF and ARF inhibited significantly the formation of AGE in the BSA/fructose system after fourteen days of incubation (Figure 3C). Also, as shown in Figure 3B and D, the CE, BF and ARF exerted stronger inhibitory effect on the BSA/glucose and BSA/fructose system after the 28-day incubation period at both doses tested.

Effect of crude extract and n-*BuOH and aqueous residual fractions on the disaccharidases*

We observed that CE, BF and ARF were able to reduce slightly maltase specific activity at the maximal dose tested (1500 µg/mL) after 5 min of *in vitro* incubation when compared with the basal group (Figure 4). The

Table 2. Acute effect of crude extract (CE), *n*-butanol fraction (BF), and aqueous residual fraction (ARF) of *M.* x *paradisiaca* on serum insulin levels (ng/mL).

Serum insulin levels in hyperglycemic rats (ng/ml)				
Time (min)	Control glucose (4 g/kg)	*M.* x *paradisiaca*		
		CE 200 mg/kg	BF 50 mg/kg	ARF 100 mg/kg
0	0.57±0.03	-	-	-
15	0.77±0.06#	0.90±0.20	1.29± 0.08**	0.88±0.15
30	0.90±0.10	0.90±0.10	0.92±0.10	0.66±0.01
60	0.54±0.04	0.71±0.08	0.72±0.15	0.73±0.10

Values are expressed as mean±SEM; n=4 in duplicate for each treatment. Statistically significant at #$p \leq 0.01$ in relation to euglycemic group. Statistically significant difference compared to the corresponding hyperglycemic group. *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

maximum effect was observed for BF which inhibited 20% of maltase activity, while this inhibitory effect was around 13% and 14% for the CE and ARF, respectively. On the other hand, none of the treatments affected the sucrase and lactase activity at any concentration tested (data not shown).

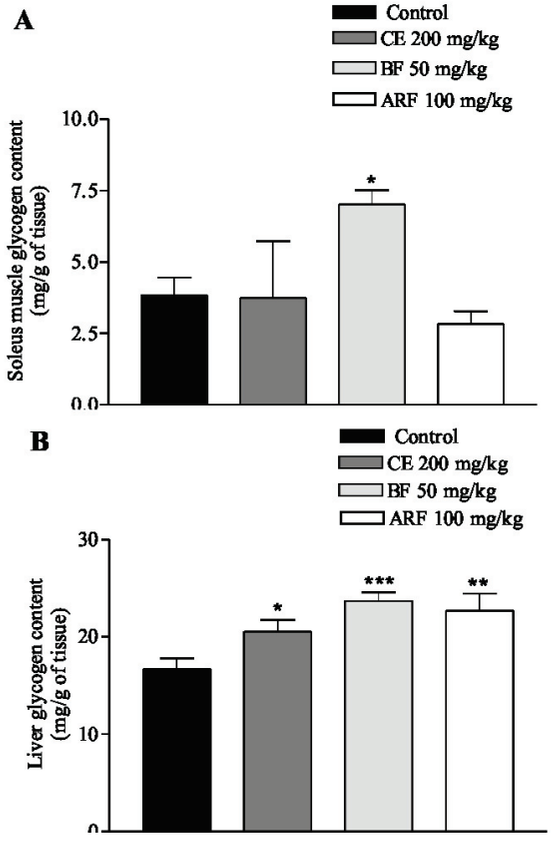

Figure 2. Effect of crude extract (CE), *n*-butanol fraction (BF) and aqueous residual fraction (ARF) and of leaves of *M.* x *paradisiaca* on the glycogen content in normal hyperglycemic rats. A. soleus muscle and B. liver 3 h after treatment by oral gavage. Control group is the hyperglycemic rats without treatment. Values are expressed as mean±SEM; n=6 in duplicate for each group. Significantly different to the corresponding hyperglycemic group; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

Discussion

In the present study, we demonstrated the beneficial effects of *M.* x *paradisiaca* leaves on the regulation of glucose homeostasis. Antihyperglycemic potency of extract and fractions, *n*-butanol and aqueous residual, was demonstrated here by glucose levels decreased, glycogen and plasma insulin levels increased after *M.* x *paradisiaca* administration in hyperglycemic rats. We have also observed that *M.* x *paradisiaca* inhibited maltase activity and the formation of AGE *in vitro*. The present study highlights the ability of *Musa* x *paradisiaca* leaves to improve carbohydrate metabolism.

The phytochemical analysis demonstrated the presence of flavonoids in crude extract and fractions of *M.* x *paradisiaca* leaves and rutin is the major compound. The presence of a variety of phenolic compounds has been reported in many parts of *Musa* species and these phytochemicals have been associated with the observed biological activity of these plants. However, to the best of our knowledge, this is the first report of the presence of rutin in the leaves of *M.* x *paradisiaca*.

Rutin (quercetin-3-*O*-rutinoside), a flavonol glycoside, is a pharmacologically active phytochemical which exhibits multiple biological activities. We have previously reported that rutin modulate glucose homeostasis. Rutin decrease glycemia, increase insulin secretion and inhibit α-glucosidase (Pereira et al., 2011, Kappel et al, 2013a,b). Also, we have demonstrated the mechanism of action of rutin in relation to glucose uptake in skeletal muscle and insulin secretion in pancreatic islets (Pereira et al., 2011, Kappel et al, 2013a,b).

Several medicinal plants show potential hypoglycemic and/or antihyperglycemic activity, including *Musa*. Intra-gastric administration of a fresh flower decoction (4 mL/kg) to hyperglycemic rabbits significantly decreased the hyperglycemic peak and/or the area under the glucose tolerance curve (Alarcon-Aguilara et al., 1998). Furthermore, it has been reported in the literature that the extracts of other parts of different *Musa* species showed a hypoglycemic effect in diabetic animal models (Ojewole & Adewunmi, 2003; Mallick et al., 2006; Adewoye et al., 2009). Pari & Maheswari (1999) observed that in a chronic daily treatment the oral administration of various doses of the chloroform extract of *M. sapientum* flowers reduced blood glucose levels in alloxanized rats. Similarly, it has been shown that pectin isolated from the juice of the inflorescence of *M. sapientum* showed significant hypoglycemic effect in alloxan-induced diabetic rats (Gomathy et al., 1990). More recently, Adewoye et al. (2009) demonstrated an antidiabetic activity for aqueous and methanolic extracts of *M. sapientum* roots in alloxan-induced diabetic rats.

Also, a hypoglycemic effect of a methanolic extract of *M.* x *paradisiaca* fruit in streptozotocin-induced diabetic mice was demonstrated (Ojewole & Adewunmi, 2003). Additionally, a composite extract of seeds of *Eugenia jambolana* and roots of *M.* x *paradisiaca* showed antihyperglycemic and anti-hyperlipidemic effect in streptozotocin-induced diabetic albino rats (Mallick et al., 2006). However all these works did not investigated the pharmacological activity as well as did not identify the bioactive compound present in the extracts of the leaves of *M.* x *paradisiaca*. In the present study, we demonstrated the significant antihyperglycemic effect of extract and fractions of *M.* x *paradisiaca* leaves.

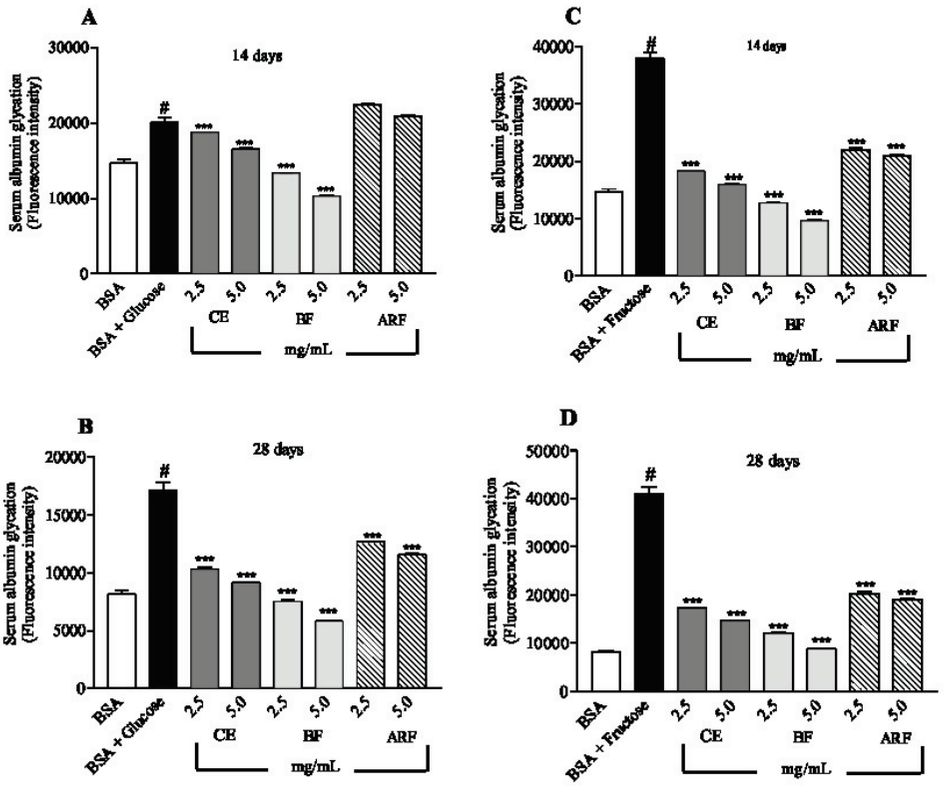

Figure 3. Inhibitory effect of crude extract (CE), *n*-butanol fraction (BF) and aqueous residual fraction (ARF) of leaves of *M.* x *paradisiaca* on the fluorescent AGE formation in a BSA/glucose or BSA/fructose system. A and B. 14 and 28-days BSA/glucose; C and D. 14 and 28-days BSA/fructose. Values are expressed as mean±SEM; n=6 in duplicate for each group. Significantly different to the corresponding control group (BSA/glucose or BSA/fructose); ***$p \leq 0.001$.

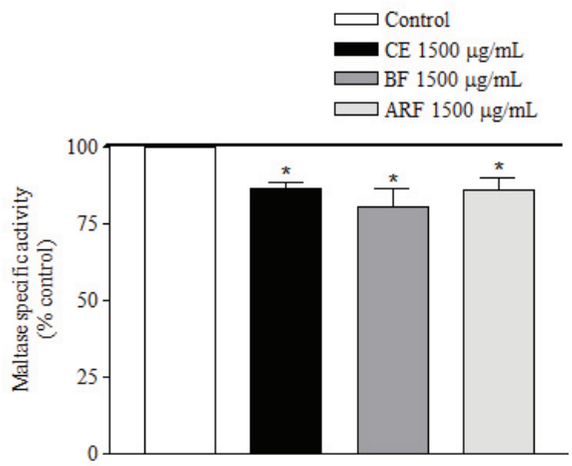

Figure 4. *In vitro* effect of crude extract (CE), *n*-butanol fraction (BF) and aqueous residual fraction (ARF) of leaves of *M.* x *paradisiaca* on specific activity of maltase, in the duodenal portion of rat intestine. Control group without treatment represents 100 % of specific activity of maltase. Incubation: 5 min. Values are expressed as mean±SEM; n=6 for each group. Significant at *$p \leq 0.05$ compared to control group.

In mammals, carbohydrate is stored mainly in the form of glycogen, with skeletal muscle and liver being the major storage sites. Glycogen metabolism is regulated by insulin/glucagon through activation and/or inhibition of several enzymes and proteins (Ferrer et al., 2003). The present results demonstrated that BF of *M.* x *paradisiaca* leaves significantly increased the glycogen content in soleus muscle and liver. The high amount of rutin into BF can be involved on increased muscle glycogen content after acute treatment, since Fernandes et al. (2010) reported that treatment with rutin was associated with a marked elevation on glycogen in rats in streptozotocin-induced diabetes. It was observed that the hepatic and cardiac tissue glycogen content increased significantly in treated diabetic rats when compared to untreated diabetic rats. Furthermore, we have demonstrated that rutin stimulated glucose uptake in the soleus muscle (Kappel et al, 2013a) and this effect could be related with the increase on muscle glycogen content with BF treatment.

Additionally, CE and ARF also showed a stimulatory effect on hepatic glycogen storage. In line with these results, it has been reported that rats fed with dietary fiber from *Musa*, showed significant lower levels of fasting blood glucose and higher concentrations of liver glycogen.

Also, the activities of some enzymes of glycolysis were inhibited (Usha et al., 1989).

The stimulation of β-cells, subsequent release of insulin and activation of the insulin receptors is a possible mechanism of natural products with potential antidiabetic activity. In the present study, it was demonstrated that BF potentiated significantly the glucose-induced insulin secretion. In this line, Folador et al. (2010) showed that the crude extract, *n*-butanol fraction and two isolated *C*-glycosylflavones, isovitexin and swertisin, of *Wilbrandia ebracteata* produced antihyperglycemic action, related to *in vivo* insulin secretion.

Additionally, flavonoids are reportedly insulin secretagogues. Genistein and daidzein have been found to increase insulin secretion stimulated by glucose *in vivo* and *in vitro* (Liu et al., 2006). Also, rutin has been reported to enhance insulin release and decrease blood glucose levels (Kamalakkannan & Prince 2006). Recently, we also have demonstrated that rutin reduce glycemia and potentiate *in vivo* insulin secretion (Kappel et al, 2013b). Thus, rutin, a potential insulin-secretagogue agent, which is found in quite high amounts in the BF, is thought to be related to the antihyperglycemic activity observed in the present study for *M.* x *paradisiaca.*

Chronic hyperglycemia and increased oxidative stress during diabetes results in the irreversible formation of AGE, which are related with development and progression of diabetic complications, like nephropathy, neuropathy, retinopathy, and cardiovascular diseases (Goh & Cooper, 2008). Our results indicated that extract and fractions of *M*. x *paradisiaca* leaves exerted stronger inhibitory effect on AGE formation. In line with these results, Bhaskar et al. (2011) also showed that the flower and pseudostem of *Musa* sp. var. elakki bale inhibited the formation of AGE in rats with streptozotocin-induced diabetes, showing the anti-AGE properties of *Musa* sp.

Also, the presence of flavonoids such as rutin may be related to inhibit the formation of AGE (Pashikanti et al., 2010), as we have demonstrated for the extract and fractions of *M.* x *paradisiaca*, particularly for the BF which presented the highest content of rutin and showed the best anti-AGE effect. In agreement with these results, anti-AGE effect was also previously reported for other flavonoids (Wu & Yen 2005).

The α-glucosidase inhibitors delay the absorption of ingested carbohydrates, reducing the postprandial glycemia and insulin peaks (De Melo & Carvalho 2006). In the present study, we demonstrated that extract and fractions of *M.* x *paradisiaca* leaves reduced the maltase activity. A number of plants are known to exert antihyperglycemic activity through the inhibition of carbohydrate-hydrolyzing enzymes in the small intestine. The polyphenols in plants play an important role in the mechanism for regulating these disaccharidases. Andrade-Cetto et al. (2008) reported that flavonoid-enriched extracts from some Mexican plants efficiently inhibited α-glucosidase activity and significantly reduced serum-glucose levels in diabetic rats. More recently, the effect of different flavonoids, including rutin, on rat intestinal disaccharidase inhibition with a consequent reduction in the intestinal glucose absorption has been described (Pereira et al., 2011).

In conclusion, we showed that the crude extract, *n*-butanol and aqueous residual fractions of *M.* x *paradisiaca* leaves exhibit potential antihyperglycemic action. The reduction on serum glucose levels, stimulation of insulin secretion, stimulation of glycogen storage, and inhibition of enzyme activity related to glucose absorption and AGE formation corroborates the beneficial effects on the regulation of glucose homeostasis observed for *M.* x *paradisiaca* leaves. In addition, *M.* x *paradisiaca* leaves that in general there is no commercial interest can provide an excellent source of rutin, which is a candidate for the development of anti-diabetic drugs in the near future.

Acknowledgements

This work was supported by grants from CNPq, CAPES, FAPESC and CAPES-REUNI. Virginia Demarchi Kappel is registered on PPGFAR-UFSC. The authors FH Reginatto and FRMB Silva are also grateful to CNPq for their research fellowships. The authors thank to Dr. Tânia Silvia Fröde for its collaboration on the insulin measurements. The authors express their appreciation to Siobhan Wiese for assistance with the English correction of the manuscript.

Author contributions

VDK (PhD student) contributed running the laboratory work, analysis of the data, drafted and revised the paper. LHC, DFP and BGP contributed to biological studies. FAM contributed to the plant extracts' preparation and chromatographic analysis. ZSB contributed to insulin serum measurements. FHR supervised the plant material identification, the phytochemical analysis as well as contributed to critical reading of the manuscript. FRMBS designed the study, supervised the laboratory work, analysis and interpretation of the data and contributed to critical reading of the manuscript. All authors have read the final manuscript and approved its submission.

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